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Nucleic Acids

III. On the Organ-Specificity of Pentosenucleic Acids

By

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Chargaff[1] claimed the organ-specificity of pentosenucleic acids (PNA) and the species-specificity of desoxypentosenucleic acids (DNA), judging from the analyses of the nitrogenous constituents in them. Concerning the composition of the former nucleic acids, the author compared those from liver of various animals within themselves and with that of pig pancreas. His analytical results of the pig pancreas PNA were pretty incoincident with Kerr and coworkers' of bull pancreas PNA.[2] But apart from this fact, it is needless to say that, in such high-molecular compounds, identity of composition does not necessarily singify identity of structure. On the other hand, the divergency of composition does not entail perfect discrepancy of structure, similar partial structures being possible to occur among pentosenucleic acids from different kinds of organ. Tokutomi,[3] one of the present workers, has already mentioned strict serological organ-specificity of the pentosenucleic acid of stomach mucosa of animals belonging to different species. This account deals with the study extended to the pentosenucleic acid of other organs, with simultaneous scrutiny of the former investigation.

Pentosenucleic acid of pig stomach (mucosa), liver and kidney (cortex) were examined regarding their precipitin reaction with anti-pig stomach (mucosa)-, anti-pig liver- and anti-pig kidney (cortex)-rabbit serum, as such as well as after cross absorption with saline extract (in vitro antigen) of those tissues. The results afforded no disproof of Tokutomi's announcement, but suggested that the liver- and kidney (cortex)-pentosenucleic acid have a common partial structure besides the probable organ-specific, although the preparations of them, employed as in vitro antigens, are not precluded to have been mixtures of two kinds of pentosenucleic acid despite of the electrophoretical homogeneity.

Experimental

Methods in General of Immunization and Precipitin Reaction

1. Antigen solutions for immunization and antisera were prepared as described in the just preceding paper from this laboratory.[3] The techniques of the precipitin test and of absorption of the antisera and the preparation procedure of tissue extracts used as in vitro antigens (Whole-tissue-antigens) also were the same as in the foregoing study.[3]

2. Pentosenucleic acids as in vitro antigens

The pentosenucleic acid of pig stomach (*mucosa*). The product by Tokutomi[1] was employed.

The pentosenucleic acid of pig liver. A fresh pig liver was cut into small pieces, freed from connective and fatty tissues as far as possible and after washing off of blood, ground into a brei, which weighed 1.53 kg. It was then stirred up in 2 *l.* of 0.14 *M* NaCl cooled with ice, and stood in an ice chest for 12 hours with occasional agitation, followed by centrifugation. The residue was extracted 3 more times. The solutions, which were strongly turbid and dark brown colored, were united and adjusted to pH 4.2 with acetic acid. A voluminous precipitate appearing thereupon (crude PNP) was centrifuged after standing overnight in an ice chest, washed with 200 cc. each of chilled 0.5 *M* NaCl and water in order to eliminate DNP and then agitated in 200 cc. of water. To the suspension a large volume of 0.5 *M* Na_2CO_3 was added to pH 7.8 and, then, to the centrifuged supernatant (yellowish-brownish and nearly clear) 10% HCl was added, under cooling, in small portions until no more precipitate occurred. Stood overnight again. The deposit was separated, washed with abs. alcohol and dried in a desiccator—**Crude PNA.** Crop, 1.02 g. Biuret intensively positive.

The product was next subjected to Sevag process:—It was taken up in 50 cc. of 10% NaCl by the aid of 10% NaOH. The solution (pH 5.6) freed from a small insoluble part was shaken vigorously with 20 cc. of a chloroform-amylalcohol (5:1 by volume) mixture for half an hour, and centrifuged. The watery layer was treated with the organic mixture 10 more times, when the gel-forming impurity scarcely remained. The final centrifuged supernatant was poured into 3 volumes of alcohol containing 0.05 *M* HCl (prepared by addition of as much conc. HCl to abs. alcohol as to obtain a 0.05 *M* HCl solution). The pentosenucleic acid came out in a gelationous form, which was centrifuged, washed with alcohol thoroughly and dried. 0.53 g. was the product here. Biuret negative.

It showed the positive Dische reaction, though weak. Hence, the

purification was attempted by precipitation of the pentosenucleic acid from a neutral solution as the barium salt, retaining the desoxypentosenucleic acid in solution, as follows:—It was dissolved in 10 cc. of water and nearly neutralized (to pH 6.8) with 10% NaOH and 5 cc. of 10% barium acetate (adjusted to pH 6.8 with acetic acid) were added. Stood overnight. The flocculent precipitate was centrifuged and after suspending in 10 cc. of water, turned into the sodium salt by adding a just sufficient quantity of sodium sulfate in small portions. The centrifuged supernatant and washing were combined and again poured into 3 volumes of the hydrochloric acid-containing alcohol. And, the gelatinous deposit was washed with alcohol and ether and dried in vacuo over $CaCl_2$ to constant. 0.29 g. of a powder tinted yellow brown was given. Biuret negative. Iodine test for glycogen also negative. Bial-Mejbaum (for pentose) strongly positive. Dische (for desoxypentose) almost negative. N (micro Kjeldahl) 13.6%, P (Haneda, Sinokawa and Yasuoka's modification of Plimmer method) 7.7%[4a], (N/P=1.8), ribose (Masamune and Ogawa's thionalide-H_2SO_4 method[5]) 36.2%, ash (Pregl method without use of H_2SO_4) 12.8%. On its electrophoresis at pH 7.8, a single boundary was demonstrated (See Fig. 1).

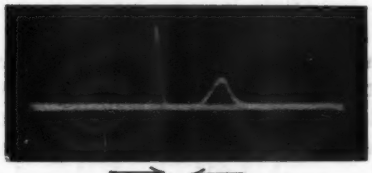

Fig. 1. Electrophoresis pattern of a 0.9% solution of pig liver pentosenucleic acid in phosphate buffer, pH 7.8, I=0.29; 13.5°C; 10 ma; exposure after starting current 40 minutes. Mobility of the boundary= $\frac{1.6\times 0.008792\times 0.3}{0.01\times 2400}=17.6$ $cm^2 \cdot sec^{-1} \cdot volt^{-1} \cdot 10^5$. Ascending limb to the left.

The pentosenucleic acid of pig kidney (cortex). A procedure differing at several stages from above was adapted.

The cortices were separated mechanically from 5 pig kidneys (0.6 kg. altogether), ground and extracted with 5 volumes of the physiological saline by shaking for 12 hours, followed by centrifugation. The brown, pretty turbid supernatant of pH 6.2 was more acidified with 10% HCl till pH 4.5. A voluminous flocculent precipitate appeared, which was separated. This and the corresponding precipitate after another run, started with 0.55 kg. material (from 6 pig kidneys), were combined and after washing with the physiological saline containing HCl, (pH 4.5),

dissolved in 300 cc. of the physiological saline by addition of 10% NaOH in drops to neutral. The centrifuged supernatant, which was somewhat turbid, was again carried down similar to above. The deposit here was taken up in 100 cc. of the physiological saline, adjusting to neutral as above, (A turbid solution was given) and after half saturation with NaCl, stood in an ice chest for 36 hours with occasional stirring. Then, immediately to the turbid solution was added 1 *N* HCl to pH 4.2, whereby a precipitate occurred. Filtered. And the nearly clear filtrate (about 90 cc.) were precipitated by addition of 3 volumes of abs. alcohol under agitation with a glass rod. Some fibrous precipitate, which adhered to the stirrer, was rejected[6] and the flocculent precipitate was collected by centrifugation and again taken up in 20 cc. of water by the aid of 1 *N* NaOH (The alkali added to pH 7.0). An opalescent solution given was reprecipitated. A fibrous precipitate less in amount came out again, which was also discarded. The major precipitate was subjected to dissolution and precipitation once more (No fibrous precipitate appeared here) and after washing with alcohol, dried. 0.79 g. of a white powder was yielded. Biuret and Dische weakly positive. Molisch and Bial-Mejbaum moderately positive. Neuberg-Saneyoshi and Goldschmiedt negative. Phosphorus and purine detected.

The balance of the crude product above (0.78 g.) was dissolved in 40 cc. of water and shaken with a mixture of 40 cc. of chloroform and 0.5 cc. of octylalcohol. The centrifuged upper layer (the solution) was separated, and treatment with the organic mixture was further repeated as long as gel formed. Finally the solution was precipitated with 3 volumes of alcohol, washed with ether and dried in vacuo over $CaCl_2$. The product (a white powder) weighed 225 mg. It showed the negative biuret, Neuberg-Saneyoshi, Goldschmiedt and Osaki-Turumi (The direct and the indirect) reaction, but the positive Molisch and Bial-Mejbaum reaction. The test with diphenylamine (Dische for desoxypentose) resulted in faint positive. Analyses: N (micro Kjeldahl) 10.9%; P (the modified Plimmer method) 6.2%; ribose (Masamune and Ogawa[5]) 28.9%; ash (micro Pregl without use of H_2SO_4) 23.2%; the proportion of N:P approaches

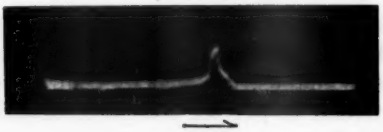

Fig. 2. Electrophoresis pattern of a 1% solution of pig kidney-cortex pentosenucleic acid in carbonate buffer, pH 9.6, I=0.12; 15°C; 10 ma; exposure after starting current 30 min. The ascending limb only is shown.

1.8:1. Electrophoresis in a carbonate buffer of pH 9.6 showed it to be homogeneous (S. Fig. 2).

Results with Comments

1. Precipitin reaction of the antisera, as such and after absorption with pig serum, with the pentosenucleic acid of the three tissues

The anti-pig liver-serum. The antiserum was prepared by injecting to a rabbit of the saline solution undialyzed of pig liver. The solution was markedly biuret and Molisch positive and moderately Bial-Mejbaum and Dische positive, and contained phosphorus and purines. The antiserum gave the precipitin reaction with the in vitro liver antigen (prepared without dialysis), as is illustrated in Table I, namely, it possessed antigen and antibody titre of 1000×2^5 and 2^5 respectively before absorption with pig serum and those of 1000×2^4 and 2^5 respectively after the absorption. The three pentosenucleic acids behaved towards the antiserum as is shown in Table II.

TABLE I

Precipitin Reaction of the Anti-Pig Liver-Rabbit Serum with the Liver in vitro Antigen

−: negative reaction, ±: faint r., +: definitely positive r.

Dilution of antiserum (n in $\times2^n$)	Dilution of reactionogen (n in $\times1000\times2^n$)								
	0	1	2	3	4	5	6	7	∞
A. The antiserum not absorbed									
0	+	+	+	+	+	+	−	−	−
1	+	+	+	+	+	+	−	−	−
2	+	+	+	+	+	+	−	−	−
3	+	+	+	+	+	+	−	−	−
4	+	+	+	+	+	−	−	−	−
5	+	+	+	+	−	−	−	−	−
6	±	±	−	−	−	−	−	−	−
B. The antiserum absorbed with pig serum									
0	+	+	+	+	+	−	−		−
1	+	+	+	+	+	−	−		−
2	+	+	+	+	+	−	−		−
3	+	+	+	+	−	−	−		−
4	+	+	+	+	−	−	−		−
5	+	+	+	−	−	−	−		−
6	−	−	−	−	−	−	−		−

TABLE II

Precipitin Reaction of the Anti-Pig Liver-Rabbit Serum with the Pentosenucleic Acids

−: negative reaction, ±: faint r., +: definitely positive r.

Reactionogen (PNA) (name of the tissue)	Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogens (n in $\times 100 \times 2^n$)				
		0	1	2	3	∞
Liver	**A. The antiserum not absorbed**					
	0	+	+	−		−
	1	+	+	−		−
	2	+	+	−		−
	3	+	+	−		−
	4	+	+	−		−
	5	+	+	−		−
	6	+	+	−		−
	7	−	−	−		−
	control*	−	−	−		−
	B. The antiserum absorbed with pig serum					
	0	+	+	−		−
	1	+	+	−		−
	2	+	+	−		−
	3	+	+	−		−
	4	+	+	−		−
	5	+	+	−		−
	6	+	+	−		−
	7	−	−	−		−
	control*	−	−	−		−
Kidney (cortex)	**A. The antiserum not absorbed**					
	0	+	+	+	−	−
	1	+	+	+	−	−
	2	+	+	+	−	−
	3	+	+	+	−	−
	4	+	+	+	−	−
	5	±	±	+	−	−
	6	−	−	−	−	−
	control*	−	−	−	−	−
	B. The antiserum absorbed with pig serum					
	0	+	+	±	−	−
	1	+	+	±	−	−
	2	+	+	±	−	−
	3	+	+	±	−	−
	4	+	+	±	−	−
	5	−	−	±	−	−
	6	−	−	−	−	−
	control*	−	−	−	−	−
Stomach (mucosa)	**A. The antiserum not absorbed**					
	0	−	−	−	−	−
	1	−	−	−	−	−
	control*	−	−	−	−	−
	B. The antiserum absorbed with pig serum					
	0	−	−	−	−	−
	1	−	−	−	−	−
	control*	−	−	−	−	−

* The antiserum was substituted with normal rabbit serum.

The anti-pig kidney (cortex)-serum. The antigen solution for immunization proved similar qualitatively as the foregoing. The antigen and antibody titre in precipitin reaction of the antiserum with the kidney in vitro antigen the tissue extract after dialysis are seen from Table III, and the reactions of the antiserum, absorbed with pig serum, with the pentosenucleic acids in Table IV.

TABLE III

Precipitin Reaction of the Anti-Pig Kidney (Cortex)-Rabbit Serum with the Kidney (Cortex) in vitro Antigen

−: negative reaction, ±: faint r., +: definitely positive r.

Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogen (n in $\times 1000 \times 2^n$)							
	0	1	2	3	4	5	6	∞
A. The antiserum not absorbed								
0	+	+	+	+	+	+	−	−
1	+	+	+	+	+	+	−	−
2	+	+	+	+	+	+	−	−
3	+	+	+	+	+	±	−	−
4	+	+	+	+	±	−	−	−
5	+	+	+	+	−	−	−	−
6	−	−	−	−	−	−	−	−
B. The antiserum absorbed with pig serum								
0	+	+	+	+	−	−	−	−
1	+	+	+	+	−	−	−	−
2	+	+	+	+	−	−	−	−
3	+	+	+	−	−	−	−	−
4	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−

The anti-pig stomach (mucosa)-serum. The antigen solution for immunization was prepared by dialysis of the tissue solution. It resembled the corresponding liver antigen solution chemical-qualitatively but was only faintly Dische-positive. The antiserum reacted with the in vitro stomach antigen and the nucleic acid of this tissue, but not with the nucleic acids from the liver and kidney (S. Tables V & VI).

In short, the pentosenucleic acid of the liver and kidney were inert to the anti-stomach serum, and that of the stomach to the anti-liver and anti-kidney serum as well, indicating that the stomach-pentosenucleic acid is not related with either of the liver- and kidney-nucleic acid at all in the present serological aspect. Contrariwise, there occurred cross reactions not only between the kidney nucleic acid and the anti-liver serum but also in the reversed case. So the examination was extended to these antisera cross-absorbed.

2. Precipitin reaction of the anti-liver serum, absorbed with pig

TABLE IV

Precipitin Reaction of the Anti-Pig Kidney (Cortex)-Rabbit Serum with the Pentosenucleic Acids

−: negative reaction, ±: faint r., +: definitely positive r.

Reactionogen (PNA) (name of the tissue)	Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogens									
		n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$					
		0	1	2	3	0	1	2	3	4	∞
Kidney (cortex)	**A. The antiserum not absorbed**										
	0	+	+	+	+	+	+	+	+	--	−
	1	+	+	+	+	+	+	+	+	−	−
	2	+	+	+	+	+	+	+	+	−	−
	3	+	+	+	+	+	+	+	+	−	−
	4	+	+	+	+	+	+	+	+	--	−
	5	−	−	−	−	−	−	--	−	−	−
	control*	−	−	−	−	−	−	−	−	--	−
	B. The antiserum absorbed with pig serum										
	0	+	+	+	+	+	+	+	±	−	−
	1	+	+	+	+	+	+	+	±	−	−
	2	+	+	+	+	+	+	+	±	−	−
	3	+	+	+	+	+	+	+	±	−	−
	4	+	+	+	+	+	+	±	−	−	−
	5	−	−	−	−	−	−	−	−	−	−
	control*	−	−	−	−	−	−	−	−	−	−
Liver	**A. The antiserum not absorbed**										
	0	+	+	−							−
	1	+	+	−							−
	2	+	±	−							−
	3	+	±	−							−
	4	+	−	−							−
	5	−	−	−							−
	control*	−	−	−							−
	B. The antiserum absorbed with pig serum										
	0	+	+	−							−
	1	+	±	−							−
	2	+	±	−							−
	3	+	−	−							−
	4	+	−	−							−
	5	−	−	−							−
	control*	−	−	−							−
Stomach (mucosa)	**A. The antiserum not absorbed**										
	0	−	−	--							−
	1	−	−	--							−
	control*	−	−	−							−
	B. The antiserum absorbed with pig serum										
	0	−	−	−							−
	1	--	--	−							−
	control*	−	−	−							−

* The antiserum was replaced with normal rabbit serum.

serum and the kidney in vitro antigen, and of anti-kidney serum, absorbed with pig serum and the liver in vitro antigen, with the liver and kidney

TABLE V

Precipitin Reaction of the Anti-Pig Stomach (Mucosa)-Rabbit Serum with the Stomach (Mucosa) in vitro Antigen

−: negative reaction, ±: faint r., +: definitely positive r.

Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogen (n in $\times 1000 \times 2^n$)								
	0	1	2	3	4	5	6	7	∞
A. The antiserum not absorbed									
0	+	+	+	+	+	+	±	−	−
1	+	+	+	+	+	+	±	−	−
2	+	+	+	+	+	+	±	−	−
3	+	+	+	+	+	+	±	−	−
4	+	+	+	+	+	±	−	−	−
5	+	+	+	+	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−
B. The antiserum absorbed with pig serum									
0	+	+	+	+	+	±	−	−	−
1	+	+	+	+	+	±	−	−	−
2	+	+	+	+	+	±	−	−	−
3	+	+	+	+	+	±	−	−	−
4	+	+	+	+	+	−	−	−	−
5	−	−	±	±	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−

pentosenucleic acid respectively

The anti-pig liver serum. The antiserum, as such and after absorption with pig serum, reacted with the kidney in vitro antigen diluted 1000×2^5 and 1000×2^8 times respectively (Table VII (A, B)). For complete absorption of 1 cc. of the antiserum, absorbed with pig serum, 30/4 mg. of the kidney extract were requested. The antiserum thus absorbed gave the precipitin reaction with the in vitro antigen and pentosenucleic acid of the liver as is shown in Table VIII. The antigen titre in the reaction with the liver nucleic acid here was 100×2^1 as when the antiserum without the absorption or after absorption with pig serum alone was applied (Cf. Table II). It is probably because the antiserum was too weak to reveal the actual decrease of the titre.

The anti-pig kidney serum. The results of the assay of the liver in vitro antigen with the antiserum, as such and after absorption with pig serum, are embodied in Table IX, and those of the assay of the kidney and liver nucleic acid with the antiserum after absorption with pig serum and the liver in vitro antigen (30/4 mg. were necessary for complete absorption of 1 cc. of the antiserum) in Table X. The antigen titre in the reaction with the kidney pentosenucleic acid was found distinctly decreased after the absorption with the liver in vitro antigen (decreased from 1000×2^2 to 100×2^2) (Cf. Table IV), but absorption even with 60 mg. of the

TABLE VI

Precipitin Reaction of the Anti-Pig Stomach (Mucosa)-Rabbit Serum with the Pentosenucleic Acids

−: negative reaction, ±: faint r., +: definitely positive r.

Reactionogen (PNA) (name of the tissue)	Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogens										
		n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$						
		0	1	2	3	0	1	2	3	4	5	∞
Stomach (mucosa)	**A. The antiserum not absorbed**											
	0	+	+	+	+	+	+	+	+	+	−	−
	1	+	+	+	+	+	+	+	+	+	−	−
	2	+	+	+	+	+	+	+	+	+	−	−
	3	+	+	+	+	+	+	+	+	+	−	−
	4	−	−	+	+	+	+	+	+	+	−	−
	5	−	−	−	−	−	−	−	+	−	−	−
	control*	−	−	−	−	−	−	−	−	−	−	−
	B. The antiserum absorbed with pig serum											
	0	+	+	+	+	+	+	+	+	+	−	−
	1	+	+	+	+	+	+	+	+	+	−	−
	2	+	+	+	+	+	+	+	+	+	−	−
	3	+	+	+	+	+	+	+	+	±	−	−
	4	−	−	+	+	+	+	+	−	−	−	−
	5	−	−	−	−	−	−	−	−	−	−	−
	control*	−	−	−	−	−	−	−	−	−	−	−
Liver	**A. The antiserum not absorbed**											
	0	−	−	−								−
	1	−	−	−								−
	control*	−	−	−								−
	B. The antiserum absorbed with pig serum											
	0	−	−	−								−
	1	−	−	−								−
	control*	−	−	−								−
Kidney (cortex)	**A. The antiserum not absorbed**											
	0	−	−	−								−
	1	−	−	−								−
	control*	−	−	−								−
	B. The antiserum absorbed with pig serum											
	0	−	−	−								−
	1	−	−	−								−
	control*	−	−	−								−

* The antiserum was replaced with normal rabbit serum.

liver in vitro antigen did no more lower the titre.

Taken altogether, the pentosenucleic acids of liver and of kidney are partially common in antigen structure.

SUMMARY

1. Electrophoretically homogeneous pentosenucleic acids of pig

liver and of pig kidney (cortex) were prepared.

2. Those pentosenucleic acids did not give the positive precipitin

TABLE VII

Precipitin Reaction of the Anti-Pig Liver-Rabbit Serum with the Pig Kidney (Cortex) in vitro Antigen

−: negative reaction, ±: faint r., +: definitely positive r.

Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogen (n in $\times 1000 \times 2^n$)							
	0	1	2	3	4	5	6	∞
A. The antiserum not absorbed								
0	+	+	+	+	+	+	−	−
1	+	+	+	+	+	+	−	−
2	+	+	+	+	+	+	−	−
3	+	+	+	+	+	+	−	−
4	+	+	+	+	+	+	−	−
5	+	+	±	−	−	−	−	−
6	+	+	−	−	−	−	−	−
7	−	−	−	−	−	−	−	−
B. The antiserum absorbed with pig serum								
0	+	+	+	+	±	−	−	−
1	+	+	+	±	−	−	−	−
2	+	+	±	−	−	−	−	−
3	+	+	−	−	−	−	−	−
4	+	±	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−

TABLE VIII

Precipitin Reaction of the Anti-Pig Liver-Rabbit Serum, double absorbed with Pig Serum and Pig Kidney (Cortex) in vitro Antigen,* with the Pig Liver in vitro Antigen and Pentosenucleic Acids

−: negative reaction, ±: faint r., +: definitely positive r.

Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogens								
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$				
	0	1	2	3	0	1	2	3	∞
A. Reactionogen: the pig liver in vitro antigen									
0	+	+	+	+	+	+	±	−	−
1	+	+	+	+	+	+	±	−	−
2	+	+	+	+	+	+	±	−	−
3	+	+	+	+	+	±	±	−	−
4	+	+	+	+	+	−	−	−	−
5	+	+	+	+	−	−	−	−	−
6	+	+	+	−	−	−	−	−	−
7	−	−	−	−	−	−	−	−	−

TABLE VIII—*Concluded*

Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogens								
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$				
	0	1	2	3	0	1	2	3	∞
B. Reactionogen: the pig liver nucleic acid									
0	+	+	−						−
1	+	+	−						−
2	+	+	−						−
3	+	+	−						−
4	+	+	−						−
5	+	+	−						−
6	+	±	−						−
7	−	−	−						−
C. Reactionogen: the pig kidney nucleic acid									
0	−	−	−						−
1	−	−	−						−

* 30 mg. of the pig kidney (cortex) in vitro antigen were requisite for complete absorption of 4 cc. of the antiserum absorbed with pig serum.

TABLE IX

Precipitin Reaction of the Anti-Pig Kidney (Cortex)-Rabbit Serum with the Pig Liver in vitro Antigen

−: negative reaction, ±: faint r., +: definitely positive r.

Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogen (n in $\times 1000 \times 2^n$)						
	0	1	2	3	4	5	∞
A. The antiserum not absorbed							
0	+	+	+	+	+	−	−
1	+	+	+	+	±	−	−
2	+	+	+	+	±	−	−
3	+	+	+	+	±	−	−
4	+	+	+	±	−	−	−
5	±	−	−	−	−	−	−
6	−	−	−	−	−	−	−
B. The antiserum absorbed with pig serum							
0	+	+	+	+	−	−	−
1	+	+	+	+	−	−	−
2	+	+	+	+	−	−	−
3	+	+	+	+	−	−	−
4	+	+	+	±	−	−	−
5	−	−	−	−	−	−	−

reaction with the anti-pig gastric mucosa-rabbit serum, and on the other hand, the pig stomach (mucosa) pentosenucleic acid did not react with the anti-pig liver and anti-pig kidney-rabbit serum.

TABLE X

Precipitin Reaction of the Anti-Pig Kidney (Cortex)-Rabbit Serum, absorbed with Pig Serum and Pig Liver in vitro Antigen, with the Pig Kidney (Cortex) in vitro Antigen and Pentosenucleic Acids

30 mg. of the pig liver in vitro antigen were used for complete absorption of 4 cc. of the antiserum. −: negative reaction; ±: faint r., +: definitely positive r.

Dilution of antiserum (n in $\times 2^n$)	Dilution of reactionogens							
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$			
	0	1	2	3	0	1	2	∞
A. Reactionogen: the pig kidney in vitro antigen								
0	+	+	±	−	−	−	−	−
1	+	+	±	−	−	−	−	−
2	+	+	±	−	−	−	−	−
3	+	+	−	−	−	−	−	−
4	+	+	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−
7	−	−	−	−	−	−	−	−
B. Reactionogen: the pig kidney nucleic acid								
0	+	+	+	−	−	−		−
1	+	+	+	−	−	−		−
2	+	+	+	−	−	−		−
3	+	+	+	−	−	−		−
4	+	+	−	−	−	−		−
5	−	−	−	−	−	−		−
C. Reactionogen: the pig liver nucleic acid								
0	−	−	−	−	−	−		−
1	−	−	−	−	−	−		−

3. The pentosenucleic acid of the liver and kidney reacted with the anti-kidney and anti-liver serum, as such as well as after absorption with pig serum, each to each and moreover after absorption with liver and kidney in vitro antigen respectively, the antisera still contained a precipitin of the nucleic acid of the tissue with which immunization had been effected.

4. From these findings combined with those in the foregoing report, it might be pronounced that the pentosenucleic acids of kidney (cortex) and of liver possess a partially common and partially organ-specific antigenic structure contrary to the strictly organ-specific pentosenucleic acid of stomach (mucosa). The presumption is that the preparations of the kidney and liver nucleic acid were really uniform substances.

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from the Education Department, which enabled us to carry out this work. H. Masamune.

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Histological Studies on Human Sacral Cord

By

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One of the authors of this report, Suzuki,[1] has previously made a histological study of human lumbar cord and succeeded in arriving at many observations that contributed something in elucidating many unclarified points on the subject. In succession to the above, the authors conducted a similar research on human sacral cord and have come at many new observations as detailed hereunder. The material was taken from the same spinal cord of which Suzuki has made use of the lumbar cord, and the same Seto's silver impregnation was used throughout in staining.

1. The motor nerve cells in the sacral cord are mostly located in cornu ventrale, only a few of them being found in the medial side of pars intermedia. In the upper sacral cord, the cells are arranged in lateral and medial groups, as in the lumbar cord, but the latter group is much poorer in development than in the lower lumbar cord, and disappears altogether in the lower sacral cord.

The lateral group, like in the lumbar cord, may be divided into the ventral, middle and dorsal subgroups, but the ventral subgroup is limited in existence to the upper sacral cord, being replaced by totally dissimilar group (Fig. 1) of special cells in the lower sacral cord. The special cell group has been known as Onufrowitz's nucleus, and supposed to represent the motor center for the musculi ischiocavernosus and bulbocavernosus, and according to Marburg,[2] Pollak[3] and Bok,[4] these special cells have been found only in the 2nd sacral segment. But our observations on their size much smaller than the typical motor cells and the poor development of their processes led us to the assumption that they are vegetative in nature. The cell nucleus of these cells is rather small-sized, so they must belong to the sympathetic nerve system.

The medial group is next to the ventral group in its poor development. It decreases in the number of component cells downwards till it disappears entirely in the lower sacral cord, while the dorsal group is well-developed and is observed even in the lowermost segment. This group is probably the motor nucleus controling the muscles in the lower limbs.

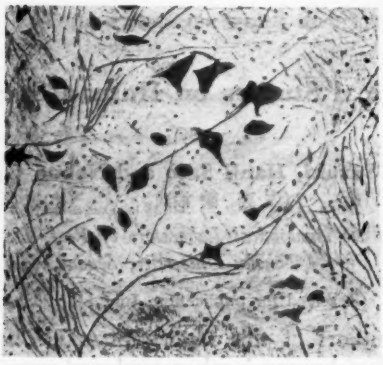

Fig. 1. Onufrowitz's nucleus in the middle part of the sacral cord. Human infant. The multiformed cells have each small-sized nucleus. Processes poorly developed. Cross-section. Seto's impregnation. X 200, reducted to ½.

The above described motor cells are similar in general form to those in the lumbar cord as reported by Suzuki.[1] Also the behavior of the incoming motor and sensory nerve fibres toward the motor cells shows no difference from that described by Suzuki concerning the lumbar cord and we see no need to repeat the description here.

2. The sensory nerve cells in zona spongiosa of the sacral cord are not much different from those in the lumbar cord. They are elongated polygonal or spindle-shaped cells arranged in 1 or 2 rows. In substantia gelatinosa, sporadic existence of sensory nerve cells is observed, but the arrangement in distinct dorsal and ventral groups grows gradually more and more blurred as the lower end of the sacral cord is approaches.

According to Waldeyer,[5] gorillas are said to have nucleus dorsalis (Clarke) in sacral cord too, but in our human specimen, we could find none of such nucleus. In cornu dorsale of the sacral cord, as in the lumbar cord, are found some sensory nerve cells in sporadic existence and their number is somewhat larger than in the lumbar cord, but these cells are generally triangular or spindle-shaped, few of them being spherical in from.

According to Kuru,[6] the cells which showed the most marked retrograde degeneration upon experimental excision of ventral and lateral tracts of the spinal cord as well as by disorder across the spinal cord were seen in the cell groups in the dorso-lateral side of the pars intermedia of the grey matter of the sacral and coccygeal cords. But he did not think them merely sensory, but sensory-vegetative, in nature.

In formatio reticularis of the sacral cord, we succeeded in discovering rather large-sized triangular or club-shaped nerve cells (Fig. 2) coming

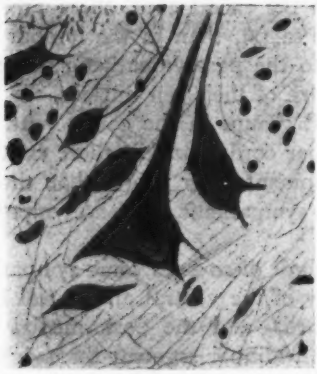

Fig. 2. 2 sensory cells and a few small parasympathetic cells found in formatio reticularis in the middle part of sacral cord in human infant. Cross-section. Same staining. X 600, reduced to 2/3.

forth in the ratio of 1–2 per horizontal section, beside the small-sized vegetative cells. The cell nucleus of the former is small, strongly stainable and always located excentrically. We could not follow up the course of the long processes. From such microscopic structures it may be inferred that they are sensory cells, and the Kuru's so-called sensory vegetative cells fall in the category of sensory nerve cells as we take it.

In the sacral cord too, the behavior of the incoming sensory fibres toward the sensory nerve cells is quite similar to that described by Suzuki[1] in lumbar cord, and we feel justified in omitting a repetition.

3. Lastly, the vegetative nerve cells in the sacral cord will be taken up. In the medial side of pars intermedia of the sacral cord, there is observed the existence of vegetative nerve cells as in the lumbar cord, but here the arrangement is sporadic and the division into the ventral, medial and dorsal groups is impossible. But here too the function of the cells is parasympathetic.

Vegetative nerve cell group corresponding to the Bok's[4] nucleus cornu commissuralis anterioris is found in the sacral cord also, which stands, however, much behind that in the lumbar cord in development. Its function is both sympathetic and parasympathetic, as in the lumbar cord.

Similarly, parasympathetic vegetative nerve cells, corresponding to the Bok's nucleus cornu commissuralis posterioris have been found in the sacral cord, much better developed than those in the lumbar cord. On the other hand, the nucleus dorsalis canalis centralis observed by Suzuki[1] in the lumbar cord was found scarcely formed in the sacral cord.

Abreast with the remarkable development of substantia gelatinosa

and cornu dorsalis in the sacral cord, there are more vegetative nerve cells contained in the sacral, than in the lumbar cord. These cells are appraised to belong to the parasympathetic nerve system, judging from their shape and the size of their cell nucleus.

In the motor cell groups in the cornu ventrale of the sacral cord, as well as of the lumbar cord (Suzuki), there exist some vegetative nerve cells, but their development is far poorer than those in the lumbar cord. Their function is both sympathetic and parasympathetic, as in the lumbar cord.

In 1908, Jacobsohn[7] discovered some sympathetic nerve cell groups at the outer boundary of the ventral and dorsal horns of the 2nd sacral segment and downwards, and the dorso-medial marginal parts of the ventral horn of the 4th lumbar segment through the 5th sacral segment, and called them nucleus sympathicus lateralis inferior and nucleus sympathicus mediosacralis respectively. Müller[8] reported the existence of parasympathetic nerve cells at the transitional parts of the ventral and dorsal horns in the 2nd through 5th sacral segments.

In confirmation of the above reports, we have succeeded in observing spindle-shaped, ellipsoid and triangular, small-sized cells in group (Fig. 3), corresponding to the nucleus sympathicus lateralis inferior of Jacob-

Fig. 3. Parasympathetic nerve cell group corresponding to the nucleus sympathicus lateralis inferior in the middle part of sacral cord. Human infant. Cross-section. Same staining. X 600, reduced to 2/3.

sohn. The number of the processes from these cells is very small and their cell nucleus is large and centrally located, so that these cells, we assumed, belong to the parasympathetic system, the opinion of Jacobsohn notwithstanding.

Jacobsohn's nucleus sympathicus mediosacralis, we found, is strongly

developed from the lumbar cord down into the sacral cord, and in the lower sacral cord, it was found to fill the gap created by the absence of motor nerve cell group. Judging from their shape, these cells are not merely sympathetic, as Jacobsohn asserts, but they comprise a majority of parasympathetic and a minority of sympathetic cells.

The grey matter in the lowermost sacral cord consists of nothing but parasympathetic nerve cells, which condition, in poorer development, extends down into the coccygeal cord. In these parts, neither motor nor sensory nerve cells are observable.

Summary

1. Motor nerve cells in the sacral cord: These are found in cornu ventrale. In the upper sacral cord, they may be divided into the lateral and medial groups, as in the lumbar cord, but the latter is poorly developed and disappears altogether in the lower sacral cord. The lateral group can be divided into the three subgroups of ventral, medial and dorsal, but the first is limited to the upper sacral cord in existence, being replaced by Onufrowitz's nucleus in the lower. This nucleus is not motor, but sympathetic in nature, histologically. The medial subgroup disappears in the lower sacral cord. On the contrary, the dorsal subgroup is well developed and reaches far down into the lowermost.

2. Sensory nerve cells in the sacral cord: In zona spongiosa, much as in the lumbar cord, elongated sensory nerve cells are seen arranged in 1 or 2 rows. There are some sensory cells scattered in substantia gelatinosa, but the distinetion between the dorsal and ventral groups is blurred in the lower sacral cord. No Clarke's nucleus was found existing. The sensory nerve cells in cornu dorsale are in sporadic existence as in the lumbar cord, but spherical cells were not found in the sacral cord. In formatio reticularis, a few vegetative cells and some rounded sensory cells were observed, the latter corresponding to Kuru's sensory-vegetative nerve cells.

3. Vegetative nerve cells in the sacral cord: In the medial side of pars intermedia are found some parasympathetic nerve cells, though the division into the three groups, as in the lumbar cord, is here impracticable. Group of sympathetic and parasympathetic nerve cells, corresponding to the Bok's so-called nucleus cornu commissuralis anterioris, and better developed group of parasympathetic nerve cells corresponding to his nucleus cornu commissuralis posterioris were also observed. Suzuki's nucleus dorsalis canalis centralis was not found in the sacral cord.

The remarkably well-developed substantia gelatinosa and cornu dorsale in the sacral cord contain a large number of parasympathetic nerve cells. In the motor nerve cell groups in cornu ventrale also are

found some sympathetic and parasympathetic nerve cells, though in a number smaller than in the lumbar cord. We found a parasympathetic cell group in the place of Jacobsohn's nucleus sympathicus lateralis inferior, and a well developed cell group in the place of Jacobasohn's nucleus sympathicus mediosacralis, the latter being composed of a majority of parasympathetic and a minority of sympathetic cells. The grey matter in the lower sacral cord contains only vegetative, in particular parasympasympathetic cells, which composition continues down into the coccygeal cord.

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Cancer Illness

among Residents of Miyagi Prefecture, Japan, 1951

By

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In Japan deaths by tuberculosis have markedly decreased these several years, while the proportion of deaths by cerebral haemorrhage and cancer (" cancer " mentioned here in this report means malignant neoplasm) has become large in the total deaths. In 1953, when the deaths of the Japanese people due to various causes were recorded by their order, cerebral haemorrhage occupied the first place, followed by cancer and tuberculosis. To make an effective cancer control, it is not sufficient to study only death cases, but, it is essential to know the status of geographical morbidity of cancer. In various foreign countries, geographical surveys on cancer patients have been conducted and the results have been published. In Japan, however, such kind of survey has not yet been conducted.

To provide such information, the Department of Public Health, Tohoku University collected the reports on cancer patients treated by physicians in whole districts of Miyagi Prefecture and tabulated the results with the cooperation of Miyagi Prefectural Government and Miyagi Prefectural Medical Association (President: Dr. Yoshihiko Koga). This survey was started with the patients seen or treated during 1951. The results of 1951 are reported here, and the results of 1952 are scheduled to be published later.

Miyagi Prefecture thus surveyed is located in the north east of Honshu (the mainland), Japan and is a coastal area of the Pacific Ocean, with the population of approximately 1.7 million and the area of 7,000 square km. City of Sendai is the political and cultural center of this prefecture. The Tohoku University is located here in Sendai. Adjacent areas to the prefecture have lower cultural level than Miyagi Prefecture. So many cancer patients of adjacent prefectures receive treatment at clinics

of this university. But few residents of Miyagi Prefecture visit hospitals of other prefectures.

In Japan, medical institutions which have twenty beds or more and can meet the definite standards prescribed are called " hopsitals " and those which have less than twenty beds are called " medical offices." In 1951, there were 88 hospitals and 731 medical offices, and the Tohoku University Hospital was the largest of all.

Survey Method

We distributed survey schedules to all hospitals and medical offices in the prefecture and required to record all the cancer patients who were seen, treated or observed for any kind of cancer and all the cancer deaths whose death certificates were made in those hospitals and medical offices during the period from Jan. 1, 1951 to Dec. 31, 1951 inclusive. The schedules were returned to the Department of Public Health of Tohoku University. The schedule made provisions for recording name, address, sex, age, marital status of the patients, previous consultations by other clinics or hospitals, first subjective symptom, date, first subjective symptom appeared, first seen (in the clinic concerned), diagnosis made, and last seen for cancer, clinical diagnosis, diagnostic methods, histological diagnosis, methods of therapy, period of hospitalization, and whether alive or dead. We then made individual cards of the patients in accordance with the schedules returned. If the same patient was reported from more than one place, only one card was made to avoid counting him as two or more cases. Moreover, in order to find out deaths occurred to the patients reported and to obtain the completeness of reporting from hospitals and medical offices, members of the Department visited 15 Health Centers in this prefecture and recorded cancer deaths by the copies of death notifications of 1951 and 1952. The Department of Public Health of Tohoku University assumed the whole responsibility for the computation of the data thus collected.

Number of Cases Reported and Completeness of Reporting

A total of 2,711 cases of cancer were seen in 1951. In this survey, nonresidents accounted for nearly one-sixth of the cases seen. The proportion of cases seen during the study year that the first subjective symptom had appeared in earlier years—approximately two-fifths (Table I).

Of the 1,061 cases of cancer among male residents seen during 1951, 35 per cent had presented their first subjective symptoms during a prior year; of the 1,201 cases among female residents, 45 per cent had presented the first subjective symptom prior to 1951.

By checking all death certificates on which cancer is recorded in the

TABLE I

Cases of Cancer First Subjective Symptom Appeared During and Prior to the Study Year, 1951

	Total	First subjective symptom appeared during study year		First subjective symptom appeared prior to study year
		reported cases	recorded from death certificates	
Total*	2,711	972	566	1,166
Residents				
All cases	2,262	784	566	912
Male	1,061	386	302	373
Female	1,201	398	264	539
Nonresidents				
All cases	439	187	—	252
Male	209	99	—	110
Female	230	88	—	142

* Including 10 cases unknown address and date first subjective symptom appeared.

TABLE II

Total Resident Cases First Subjective Symptom Appeared During the Study Year and Cases Recorded from Death Certificates only, by Sex and Primary Site, 1951

Primary site	Total case//	First subjective symptom appeared during study year		
		total	recorded from death certificates only	
			number	percent
All cases	2,262	1,350	566	41.9
Male	1,061	688	302	43.9
Female	1,201	662	264	39.9
Buccal cavity and pharynx	21	12	4	33.4
Digestive organ and peritoneum	1,406	929	446	48.0
Respiratory system	83	44	19	43.1
Breast	119	51	6	11.8
Female genital organs*	363	158	38	24.1
Male genital organs	18	7	3	42.9
Urinary organs	19	10	6	60.0
Skin	19	8	—	0
Other and unspecified sites†	39	18	2	11.1
Eye, brain and nervous system	12	7	1	14.3
Lymphomas‡	59	37	13	35.2
Leukemias and aleukemias	34	31	16	51.6
Sarcomas§	53	30	8	26.7
Other and unspecified malignant neoplasms	17	8	5	62.5

* Including chorionepithelioma. † Carcinoma only. ‡ Including Hodgkin's disease. § Including all sites. // Total cases, first subjective symptom appeared during or prior to the study year.

column of cause of death against the file of reported cases, it was possible in this survey to find out cancer deaths which were not reported as cases by hospitals or medical offices. In 1951, cancer deaths that had not been reported as cases accounted for 42 per cent (566 cases) of the total known resident cases. Among the total of patients, the percentage by site of the cases, not reported from hospitals or medical offices but confirmed only by death certificates are indicated in Table II.

It is the fact that in Japan, there are many cancer patients expected to die are discharged from hospitals and die at their own houses. In Miyagi Prefecture in 1949, 1950 and 1951, among the total of cancer deaths, the percentage of deaths at hospitals and medical offices was 18.8 per cent in male and 15.3 in female and the rest almost all died at their own houses. There are many physicians who are requested by the families of dead persons to visit their houses only to make death certificate and, in such case, most of these physicians are not the physicians who cared of the cancer patients.

It is presumed that many physicians who were in such condition did not report of death in this survey. For this reason, it is presumed that there will be found out a large percentage of deaths not reported in this survey.

The number of hospitals who reported survey schedules and the hospitals to which members of the Department of Public Health visited to make survey schedules was 63. That of medical offices was 176. The number of beds of hospitals from which we obtained the survey schedules corresponds to 86 per cent of the total of beds in all hospitals.

Accuracy of Reports

Each respondent was requested to indicate on the report whether the diagnosis of cancer was confirmed by a microscopic examination of tissue, operation or X-ray examination. Table III shows this information for this survey, classified by primary site of neoplasm.

Whether the diagnosis of cancer is confirmed by microscopic examination depends in part upon the accessibility of the tissue affected, especially for living cases. For example, among patients with cancer of the liver and biliary passages, an inaccessible site, 2.6 per cent of diagnosis were confirmed microscopically, while 43.2 per cent of cancer of the uterus, an accessible site, were microscopically confirmed. It seems that percentage of diagnosis by operation or X-ray examination can become reference data for accuracy of diagnosis.

Incidence, Prevalence and Mortality Rates

Incidence, prevalence and motality rates were computed based on

TABLE III

Percentages of Reported Cases of Cancer with Microscopic Confirmation, Operation and X-ray, by Primary Site, 1951

Primary site	Total cases reported	Microscopic confirmation		Operated cases, without mentioning microscopic confirmation		X-ray examination only	
		number	%	number	%	number	%
Total	1,515	425	28.1	352	23.2	323	21.3
Buccal cavity and pharynx	17	9	52.9	1	5.9	—	0
Digestive system	851	134	15.7	195	22.9	294	34.5
Esophagus	69	11	15.9	3	4.3	39	56.5
Stomach	598	99	16.6	157	26.3	213	35.6
Intestines	24	9	37.4	4	16.7	1	4.2
Rectum	61	13	21.4	20	32.8	4	6.5
Liver and biliary passages	78	2	2.6	6	7.2	27	34.8
Pancreas	17	—	0	3	17.7	10	58.9
Peritoneum	4	—	0	2	50.0	—	0
Respiratory system*	32	12	37.6	1	3.1	12	37.6
Accessory sinuses	23	6	26.0	3	13.0	4	17.4
Breast	105	33	31.4	54	51.4	—	0
Uterus†	257	111	43.2	66	25.7	—	0
Other female genital organs‡	35	11	31.4	10	28.6	3	8.6
Male genital organs	14	8	57.0	1	7.1	—	0
Urinary organs	11	3	27.4	2	18.2	3	27.4
Skin	16	7	43.6	3	18.7	—	0
Thyroid gland	15	11	73.4	2	13.3	—	0
Other and unspecified sites§	19	7	36.8	2	10.5	3	15.8
Eye	7	6	86.0	—	0	—	0
Brain and nervous system	5	4	80.0	—	0	—	0
Lymphomas	37	22	59.4	1	2.7	—	0
Hodgkin's disease	6	4	66.7	2	33.4	—	0
Leukemias and aleukemias	17	14	82.4	—	0	—	0
Sarcomas//	40	20	50.0	8	20.0	2	5.0
Other and unspecified malignant neoplasms	8	3	37.6	1	12.5	1	12.5

* Excluding accessory sinuses † Excluding chorionepithelioma. ‡ Including chorionepithelioma. § Carcinoma only. // Including all sites.

the figure obtained by this survey. The full value of morbidity data can be obtained only by relating the number of cases to the population from which they are drawn through the computation of rates per unit of population. The population used in computing the rates given in this report was based on the October 1, 1950 population census (though this is the previous year's population of this survey, this is the newest one officially announced). The total population of Miyagi Prefecture is enumerated at 1,663, 442 in 1950 (829,050 males and 834, 392 females). The rates were defined as follows:

1. *Incidence rate.* The number of cases whose first subjective symptom of cancer appeared during the study year, per 100,000 population. In this kind of survey in the United States, incidence rate is

computed by the number of cases first diagnosed with cancer during the study year instead of by the above mentioned definition. But in this survey, we considered it is difficult to make this date of diagnosis standard. The reasons are that it is hard to require full reports from all physicians and, in Japan, most physicians who made the first diagnosis do not tell patients as cancer cases apparently. In the survey schedule, there is an item " Date first seen with cancer " in the United States, but since there will be probability not to obtain complete answers from patients, we made an item " Date first subjective symptom appeared " in addition. The defects of our method are that in case subjective symptom first appeared at the end of the study year and first seen by a physician next year, this case will not be recorded. Accordingly the first year's study will be underestimated than the exact number. Now at present, we are studying at 1952 study year. If we finish enumeration of this 1952 year's survey, we shall be able to obtain the cases that symptom first appeared at the end of 1951 and seen by physicians in 1952.

2. *Prevalence rate.* The number of cases known to have had cancer at any time during the study year, per 100,000 population. (The patients who visited physicians for diagnosis only during the study year and did not receive any medical care were included.)

3. *Mortality rate.* The number of deaths during the study year, per 100,000 population. We considered such case that, in the death certificate, in the column of " Disease or condition directly leading to death " as a matter of course, in that of " Antecedent causes " or " Other significant conditions " also cancer is recorded as cancer death. In Japan, the copies of important columns of death certificates have been kept in Health Centers since 1948.

The number of resident cases of cancer in 1951 in the prefecture collected from this survey and death certificates is 2,262, 47 per cent in male and 53 percent in female. The items of 2,262 resident cases are as follow: 1,350 persons (60 percent of total cases) were cases whose first subjective symptom appeared in 1951, including 566 deaths revealed by death certificates only (we counted backward from the interval between onset and death recorded on death certificates to determine when the symptom first appeared in the death cases not reported from physicians); 912 cases (40 per cent of total) presented their first symptom during 1950 or in previous year and received medical care or visited physicians for only being observed their process during 1951.

Thus, 81 out of every 100,000 persons living in Miyagi Prefecture had first subjective symptom of cancer during the study year, and 136 out of every 100,000 persons were under treatment or under observation for cancer during this period (Table IV).

TABLE IV

Cancer Incidence, Prevalence and Mortality Rates per 100,000 Population, by Sex and Primary Site, 1951

Primary site	Incidence rate			Prevalence rate			Mortality rate		
	Both sexes	Male	Female	Both sexes	Male	Female	Both sexes	Male	Female
Total	81.2	83.0	79.3	136.0	127.9	143.9	75.4	78.6	72.1
Buccal cavity and pharynx	0.7	0.8	0.6	1.3	1.3	1.2	0.3	0.4	0.2
Lip	0.1	0.1	0.1	0.4	0.5	0.2	0.1	0.1	0
Tongue	0.4	0.5	0.2	0.5	0.5	0.6	0.1	0.1	0.1
Other mouth	0	0	0	0.1	0.1	0	0	0	0
Pharynx	0.2	0.2	0.2	0.3	0.2	0.4	0.1	0.1	0.1
Digestive organs and peritoneum	55.8	68.0	43.7	84.5	102.8	66.4	54.1	65.0	43.3
Esophagus	4.5	5.7	3.4	6.7	8.7	4.8	4.9	6.3	3.6
Stomach	35.7	45.7	25.8	56.3	71.4	41.2	34.6	43.4	25.9
Intestines	1.9	1.9	1.9	2.7	2.8	2.6	1.6	1.7	1.6
Rectum	2.4	2.2	2.6	5.0	4.9	5.0	2.0	2.2	1.9
Liver and biliary passages	9.8	11.2	8.4	11.7	13.1	10.2	9.5	10.0	9.0
Pancreas	1.1	1.1	1.2	1.4	1.3	1.6	0.8	0.8	0.7
Peritoneum	0.3	0.1	0.5	0.7	0.4	1.0	0.5	0.5	0.6
Other digestive	0.1	0.1	0	0.1	0.1	0	0.1	0.1	0
Respiratory system	2.6	3.6	1.7	5.0	6.0	4.0	2.8	3.5	2.0
Accessory sinuses	0.7	1.1	0.4	1.9	1.9	1.9	0.8	0.8	0.8
Larynx	0.5	0.5	0.5	1.0	1.4	0.6	0.4	0.6	0.2
Bronchus and lung	1.1	1.7	0.6	1.6	2.2	1.0	1.2	1.7	0.7
Pleura	0.3	0.4	0.2	0.4	0.4	0.5	0.2	0.2	0.2
Mediastinum	0	0	0	0.1	0.1	0	0.1	0.1	0
Breast	3.1	0.2	5.9	7.2	0.4	13.9	1.3	0	2.5
Female genital organs	9.5	...	18.9	21.8	...	43.5	8.0	...	15.9
Uterus	8.1	...	16.2	19.4	...	38.7	7.0	...	13.9
Cholionepithelioma	0.7	...	1.4	1.1	...	2.2	0.5	...	1.0
Ovary	0.4	...	0.8	0.7	...	1.4	0.5	...	1.1
Other female genital	0.2	...	0.5	0.6	...	1.2	0		0
Male genital organs	0.4	0.8	...	1.1	2.2	...	0.4	0.8	...
Prostate	0.1	0.2	...	0.4	0.7	...	0.2	0.5	...
Testis	0.2	0.4	...	0.4	0.7	...	0	0	...
Other male genital	0.1	0.2	...	0.4	0.7	...	0.2	0.4	...
Urinary organs	0.6	0.8	0.4	1.1	1.4	0.8	0.5	0.6	0.5
Kidney	0.1	0.2	0	0.3	0.5	0.1	0.1	0.1	0
Bladder	0.5	0.6	0.4	0.8	1.0	0.7	0.4	0.5	0.2
Other urinary	0	0	0	0	0	0	0.1	0	0.2
Skin	0.5	0.5	0.5	1.1	1.0	1.3	0.2	0.1	0.2
Thyroid gland	0.5	0.4	0.6	0.9	0.5	1.3	0.2	0.2	0.1
Other and unspecified sites*	0.6	0.5	0.7	1.4	1.0	1.9	0.9	0.8	1.0
Eye	0.3	0.5	0.1	0.4	0.7	0.1	0.1	0.1	0
Brain and nervous system	0.1	0.1	0.1	0.3	0.4	0.2	0	0	0
Lymphatic and haematopoietic tissues	4.1	4.8	3.4	5.6	6.4	4.8	4.0	4.6	3.5
Lymphomas	1.8	2.1	1.6	2.8	3.1	2.5	1.7	1.9	1.4
Hodgkin's disease	0.4	0.5	0.4	0.7	1.0	0.5	0.5	0.7	0.4
Leukemias and aleukemias	1.9	2.3	1.4	2.0	2.3	1.8	1.8	1.9	1.7
Sarcoma, all sites	1.8	1.2	2.4	3.2	2.8	3.6	1.7	1.4	1.9
Other and unspecified malignant neoplasms	0.5	0.6	0.4	1.0	1.2	0.8	1.0	1.0	1.0

* Carcinoma only.

There were 1,254 resident deaths in 1951 for cancer, a mortality rate of 75 per 100,000 population.

The recorded cancer incidence rate among males exceeded that for females by about 5 per cent (83.0 compared to 79.3). But in prevalence rate, the relation is opposite. The female cancer prevalence rate exceeded the male rate by about 12 per cent (143.9 compared to 127.9).

This apparent difference is a reflection of the large number of cases of uterus and breast cancer among female.

Male incidence rates were greater than those of the females for cancer of the esophagus, stomach, and bronchus and lung. The incidence rate of bronchus and lung cancer for males was three times as great as that for females.

In cancer mortality, the male rate was 9 per cent greater than the female rate (79 and 72 per 100,000).

Survival Rates

Data on resident cases of cancer newly diagnosed in 1951 were analyzed with respect to survival after diagnoses. The basic information on survival was obtained by checking the names of reported cases against death certificates for the period January 1, 1951—December 31, 1952.

Fifty-two out of every 100 cases newly diagnosed in 1951 survived 12 or more months after diagnosis while 63 out of every 100 survived at

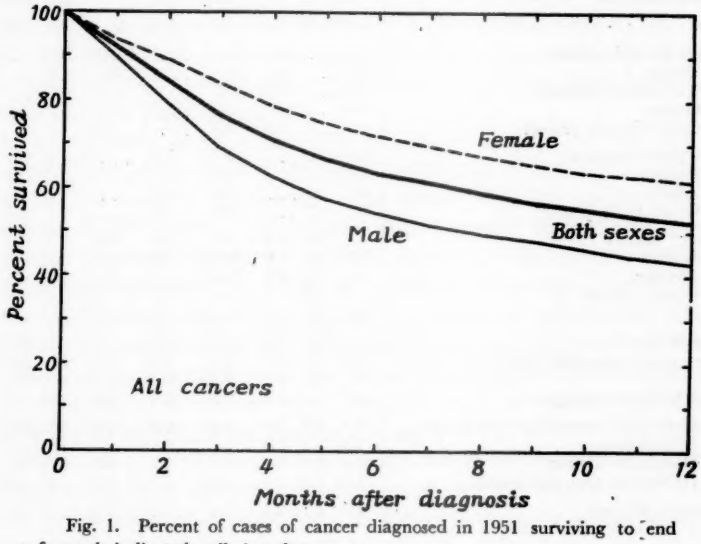

Fig. 1. Percent of cases of cancer diagnosed in 1951 surviving to end of month indicated; all sites, by sex

TABLE V

Six- and Twelve-month Survival Rates for Cases of Cancer Newly Diagnosed during 1951, by Sex, Age and Primary Site

Sex, age and primary site	Number of cases diagnosed	6-month survival		12-month survival	
		rate	standard error	rate	standard error
All cases	1,211	0.63	0.01	0.52	0.01
Male	592	.54	.02	.43	.02
Female	619	.72	.02	.61	.02
Selected primary sites:					
Digestive system	725	.55	.02	.44	.02
Stomach	506	.54	.02	.42	.02
Male	336	.53	.03	.41	.03
Female	170	.57	.04	.43	.04
Operated cases	193	.70	.03	.55	.04
Non-operated cases	313	.45	.03	.34	.03
Esophagus	59	.42	.06	.34	.06
Liver and biliary passages	75	.43	.06	.39	.06
Respiratory system	46	.67	.07	.52	.07
Breast (female only)	58	.97	.02	.91	.04
Female genital organs	204	.86	.03	.74	.03
Uterus*	179	.87	.03	.75	.03
Operated cases	55	.91	.04	.84	.05
Radiotherapy only	56	.89	.04	.79	.05
Not mentioned, operation or radiotherapy	68	.81	.05	.65	.06
Lymphatic and haematopoietic tissues	52	.32	.06	.23	.06
Age groups:					
Under 30 years	71	.58	.06	.46	.06
30 years—39 years	82	.77	.05	.71	.05
40 years—49 years	225	.73	.03	.64	.03
50 years—59 years	365	.64	.03	.52	.03
60 years—69 years	329	.60	.03	.47	.03
70 years and over	134	.46	.04	.38	.04

* Excluding cholionepithelioma.

least 6 months. In other words, 5 out of every 10 patients died within 1 year of diagnosis, but 4 of these died during the first 6 months. This relationship reflects the relatively high death rate among cancer patients during the first few months after diagnosis. The survival rates given in Table V and in Figs. 1 and 2.

The variation in survival rates naturally depends on stage at diagnosis. In this survey, we requested recording on stage at diagnosis. But we got little recording of accuracy.

Survival rates varied appreciably by sex. The male 12-month survival rate was 43 compared to 61 for female. The differences in survival rates among both sexes are mainly influenced by the cases of the cancer of the uterus and the breast.

Among the various sites, the lowest rates were found among lymphomas (including Hodgkin's disease), leukemias and aleukemias (23 percent

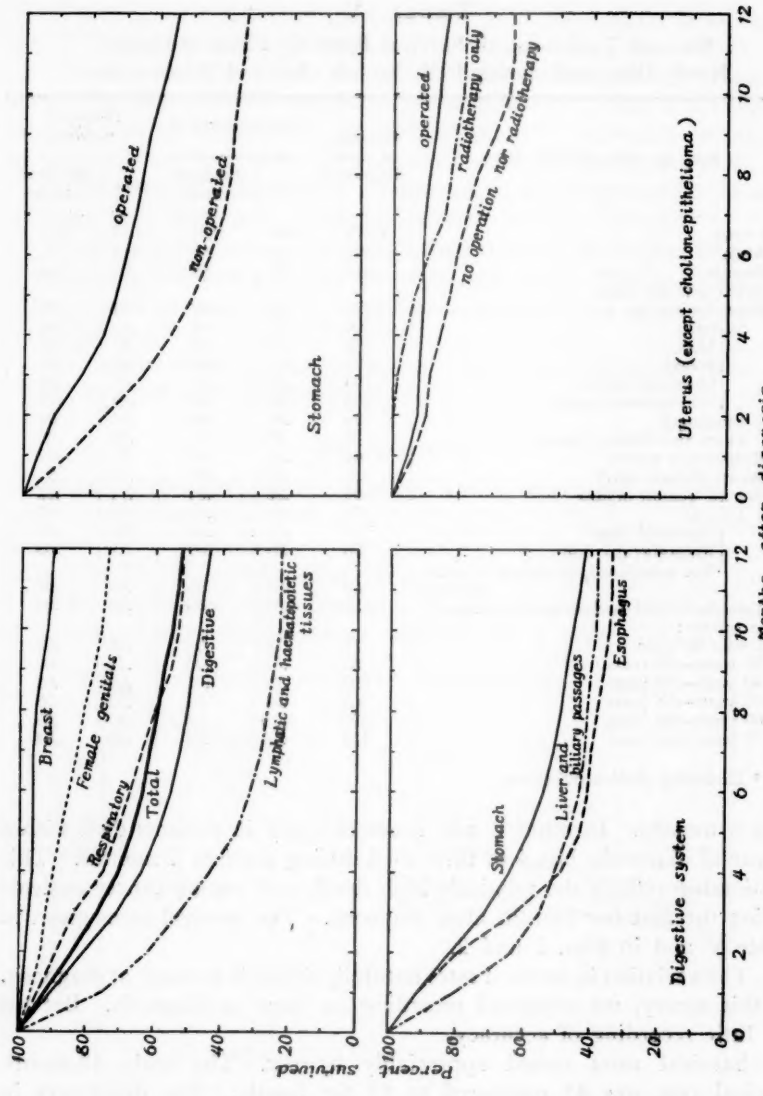

Fig. 2. Percent of cases of cancer diagnosed in 1951 surviving to end of month indicated; selected sites

survived 1 year, and 32 per cent survived 6 months), esophagus (34 per cent and 42 per cent), and liver and biliary passages (39 per cent and 43 per cent). These may be compared with rates of 91 per cent survived 1 year and 97 per cent survived 6 months for the breast.

We computed survival rate on cancer of the stomach and uterus according to that whether operation was made or not. Viewing the results, in stomach cancer, if operated, 12-month survival rate is 55 per cent and if not operated, 34 per cent. In uterus cancer, if operated, 12-month survival rate is 84 per cent. In case only radiotherapy was given, 79 per cent. In case of no operation nor radiotherapy recorded, 12-month survival rate is 65 per cent.

Summary

1. We distributed survey schedules to all the hospitals and medical offices in Miyagi Prefecture, Japan which has about 1,663,000 population and collected reports on all the cancer patients seen or treated during 1951. At the same time, we studied at the cases that cancer is recorded as cause of death out of the copies of the death notifications kept in all the Health Centers in the prefecture. The results obtained by the above mentioned two kinds of survey were enumerated.

2. The case number reported from hospitals and medical offices was 2,804 in total. However, since one patient is likely reported from two or more of hospitals and/or medical offices, the exact number was 1,964. Among the patients, the number of residents of Miyagi Prefecture is 1,515. As there are no any other hospitals equal to or larger than the Tohoku University Hospital in scale in adjacent prefectures, many persons visit this hospital from adjacent prefectures. It is presumed that few residents of Miyagi Prefecture ask to have examination and treatment to hospitals in other prefectures.

The number of such cases that "cancer" was recorded in any column of cause of death in the death certificates was 1,254 (male 652, female 602). Among these cases, 507 were reported from hospitals and medical offices. In 1951, out of residents of Miyagi Prefecture, the exact number of known cancer cases was 2,262 (male 1,061, female 1,201). Among them, 1,350 presented first subjective symptom in the study year.

3. An incidence rate of cancer patients who felt their first subjective symptom in 1951 was 81 (male 83, female 79) per 100,000 population. A prevalence rate of all the cancer cases, cancer deaths and cancer cured cases that physicians dealt with for some reasons in the study year was 136 (male 128, female 144) per 100,000 population. A mortality rate of the cases that cancer were recorded in any column of cause of death in the death certificates was 75 (male 79, female 72) per 100,000 population.

4. Viewing incidence cases by site, in male stomach (55% of total male incidence cases), liver and biliary passages (14%), and esophagus (7%) show large number in frequency. Leukemias and aleukemias, rectum, lymphomas, intestine, and lung succeed the former, but they

have a small proportion. In female, stomach (32% of total female incidence cases), uterus (21%), liver and biliary passages (11%), and breast (7%), esophagus, rectum, and sarcomas succeed the former. Skin and prostate cancer occurs markedly in small frequency.

5. The 12-month survival rate of newly diagnosed patients in 1951 is male, 43 per cent and female, 61 per cent. In stomach cancer male, 41 per cent and female, 43 per cent after twelve months and male, 53 per cent and female, 57 per cent after six months. Comparing a survival rate of a group of patients whose stomach and uterus cancer was operated to that of non-operated group of patients, it was recognized that a survival rate of the former is higher than that of the latter.

This work was carried out by a Grant from the Foundation for Scientific Research of the Ministry of Education.

M. Segi

References

1) National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency, U.S.A., Cancer Morbidity Series, 1950–1952 (Public Health Service Publication Nos. 13, 65, 67, 112, 126, 152, 178, 216, 217, 244).

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Chemistry and Biology of Lipids

XV. On Group Lipoid from Human Liver

Third Report

By

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The group lipoid was separated from the liver of persons belonging to Group A by a procedure substantially same as that reported by Matuda,[1] but, since the product proved electrophoretically inhomogeneous, it was purified with Ba-acetate and chloroform. The whole preparation procedure and some properties of the final product will be described in this account.

Experimental

A batch of tissue lumps weighing 2948 g. of liver of Group A persons was chopped and after short washing with physiological saline on a linen cloth, kept under 5 volumes of 95% alcohol for 1 week. Then it was squeezed, ground in a masticator and dried in a Faust apparatus.

Of the material thus obtained (623 g.), a half was taken for one run: —It was boiled under reflux with 5 volumes of abs. alcohol (1550 cc.) for 3 hours, and filtered while hot. The residue was extracted once more with 3 volumes of the alcohol in the same way. And the alcoholic solutions (2510 cc. in all) were combined, and stood overnight in an ice-chest. The yellowish deposit—Fr. I (Sphingolipid fr. I)—was collected on a Buchner funnel and washed well with the alcohol, and the brown filtrate and washing (somewhat fluorescent) were concentrated together under diminished pressure. The brown sticky still-residue (40.5 g.) was dried overnight in a vacuum desiccator, and then kneaded with 200 cc. portions of acetone, until no more browning of this solvent occurred. The acetone solutions (800 cc.) were vacuum-distilled. The remaining yellowish brown oily mass consisted of cholesterol and neutral fat—Fr. II (Cholesterol & neutral fat fr.). The tan powder after exhaustion with acetone (20.5 g.) was agitated in 150 cc. of water-containing ethylether

and centrifuged. The centrifugate was further treated with the ether again and again (five times) and finally with acetone, and dried in vacuo —Fr. III (Sphingolipid fr. II). The ethereal solutions united (clear, brown) were evaporated to a thick solution, precipitated with a large excess of acetone, and dried in vacuo. 15.65 g. was the yield. The substance was found completely soluble in benzene. It was dissolved in 20 cc. of water-containing ether and 400 cc. of abs. alcohol added. The flocculent deposit was washed with alcohol and dried as above. A white yellowish powder given amounted to 1.9 g. (Fr. IV). On evaporating the supernatant to dryness, a brown waxy mass (Lecithin & cephalin fr.) was left—Fr. V. Yield 12.5 g. Fr. IV (A small part was set aside for the serological assay) was washed with three 50 cc. portions of dry ether. The most part remained undissolved which was dried—Fr. IV′. 1.8 g. was this division. The Fr. V was again treated with abs. ether, employing three 50 cc. portions of the solvent. The centrifuged supernatant were distilled together in vacuo to dryness and after washing with abs. alcohol, dried in a vacuum desiccator over $CaCl_2$—Fr. Va (7.6 g.). The insoluble part was also dried in a similar manner—Fr. Vb. This fraction weighed 4.8 g.

Examination of their anti-isohemoagglutinative potencies (Table I) demonstrated that, of the fractions above, IV and IV′ contained the group lipoid. Hence, the latter was next stirred up in 50 cc. of water and the brown opalescent solution was alkalinized with 1 *N* NaOH to pH 9.6 and shaken three times with benzene, followed each time by centrifugation. The upper benzene layers, transparent and yellowish, were united and distilled to dryness—Fr. VI. The lower layer after the last operation, filled with fine foams, was acidified with 1 *N* HCl, whereby the foams turned into a transparent solution. This watery solution was saturated with NaCl and again extracted with benzene three times. The benzene solutions here were combined and also distilled to dryness, and after washing with acetone, dried. The portion given amounted to 1.5 g.—Fr. VII. It was next taken up in 50 cc. of water saturated with NaCl, and after addition of 1 *N* HCl to pH 1.2 (against T. B. & C. R.), shaken three times with chloroform. The chloroform solutions were distilled together in vacuo, washed with acetone and dried —Prep. I. Yield 1.2 g.

As is shown in Fig. 1A, the product was inhomogeneous, containing one or two contaminations, so it was subjected to purification following.

Purification. Prep. I was dissolved in 80 cc. of water. A dark brown, somewhat turbid solution was given. After addition of 10 cc. of 10% barium acetate, it was stood overnight in an ice chest, and the brownish flocculent precipitate was centrifuged, washed three times with 10%

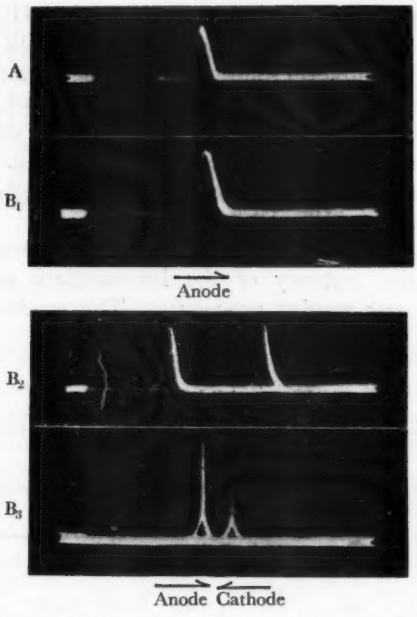

Fig. 1. Electrophoretic patterns of 1% solutions of (A) Prep. I and (B_1~B_3) Prep. II in (A and B_1) phosphate buffer of pH 7.8 and I 0.29, (B_2) acetate buffer of pH 4.6 and I 0.10 and (B_3) carbonate buffer of pH 8.9 and I 0.11. Temperature of tests were 20°, 15°, 15° and 15°C and durations of exposure 55, 55, 20 and 15 minutes respectively. Gradients of diagonal slit 35°. The ascending limb alone is shown in (A) and (B_1) and the ascending limb to the left in (B_2) and (B_3). Mobility:

(A), $\dfrac{1.75\times0.01002\times0.3}{0.01\times3300}=15.9\ \mathrm{cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot 10^5}$

(B_1), $\dfrac{1.80\times0.0096\times0.3}{0.01\times3300}=15.7$ "

(B_2), $\dfrac{\dfrac{1.25+1.30}{2}\times0.0045\times0.3}{0.01\times1200}=14.4$ "

(B_3), $\dfrac{\dfrac{1.85+1.90}{2}\times0.0031\times0.3}{0.01\times900}=19.4$ "

barium acetate and dried (Ppt. I). The yellowish supernatant and the washings were vacuum-distilled to one third the volume. And the precipitate occurring thereby was also centrifuged and washed as above (Ppt. II). The Ppts. I and II combined were suspended in 20 cc. of water, 8 cc. of the barium acetate solution added, and the centrifuged supernatant decanted out. The centrifugate was further washed repeatedly until no more staining of the washing happened. It was again suspended

in 70 cc. of water, acidified with 20 drops of 1 *N* HCl to pH 1.2, and after addition of 2 g. of barium chloride, shaken with 70 cc. portions of chloroform. The chloroform solutions were concentrated to about 100 cc. under reduced pressure. The condensate was washed twice with water, distilled to dryness and after washing with acetone, dried in vacuo over H_2SO_4. A yellowish brown powder weighing 0.98 g. was given—Prep. II. This gave only a single boundary after electrophoresis at pH's 4.7, 7.8 and 8.9. (S. Figs. $1B_1$, $1B_2$ and $1B_3$.)

Group activity, and conversion phenomenon of the group specificity. Anti-isohemoagglutinative potency of the intermediate and final products were assayed by our routine procedure. The results are embodied in Table I.

TABLE I

Anti-isohemoagglutinative Potency of Intermediate and Final Products

A and B sera had agglutinin titers 1280 and 320 respectively and diluted to 1:60 and to 1:15. h=hemolysis.

Fraction	Group of red cells	Dilution of substances (1 :)							
		50	10^2	2×10^2	4×10^2	8×10^2	1.6×10^3	3.2×10^3	6.4×10^3
Fr. I	A	h	h+	++	++	++	++		
	B	h	h+	++	++	++	++		
Fr. II	A	h	h+	h+	h+	++	++		
	B	h+	h+	h+	h+	++	++		
Fr. III	A	+	+	++	++	++	++		
	B	+	+	++	++	++	++		
Fr. IV	A	−	−	−	−	−	+		
	B	+	+	++	++	++	++		
Fr. Va	A	−	±	±	+	+	+		
	B	+	+	+	++	++	++		
Fr. Vb	A	h	h+	h+	+	+	+		
	B	h	h+	h+	+	+	+		
Fr. VI	A		±	+	+	+	++		
	B		+	++	++	++	++		
Fr. IV′	A		−	−	−	−	±	+	++
	B		+	++	++	++	++	++	++
Prep. I	A		−	−	±	+	+	++	++
	B		−	−	−	−	−	+	++
Prep. II	A		−	−	−	±	+	++	++
	B		−	−	−	−	−	±	+

It is seen from the table that Group B potency (anti-β potency), which Fr. IV′ lacked, became manifest in Prep. I, Group A potency lowering in some degree. The group conversion must have occurred either when the alkaline or acidic watery solution of Fr. IV′ was shaken with benzene or when the acidic watery solution of Fr. VII was shaken with chloroform. The conversion of blood group did not occur in the other run. The final products were ascertained to have no Group O activity.

Chmical and Physical Properties of the Final Product Prep. II

Solubilities: The pure preparation dissolved in chloroform, benzene, tetralin and pyridine completely, but was partly soluble in water-containing ether and insoluble in both hot and cold acetone as well as in abs. ether. In abs. ethanol and abs. methanol, it did not dissolve in the cold, but dissolved slightly when heated. In cold glacial acetic acid it dissolved only in part, but completely in the hot acid. In water it formed a nearly transparent solution, and alkalinization of the watery solution did not give rise to a marked change of appearance.

Qualitative tests: It gave positive Molisch test, but negative biuret, Osaki-Turumi (both the direct and indirect) and Elson-Morgan reaction. Intensive diazo test of Rosenthaler for alcohol was given.

Chemical analysis: N (micro Kjeldahl) 1.6%; P (Haneda, Sinokawa and Yasuoka's modification of Plimmer method) 4.2%; N:P=1.0:1.1; total carbohydrate as glucose (Masamune and Tanabe, First Method)[3] 2.6%.

Fluorescence. The solid substance fluorescented pale grey, its watery solution pale green and its chloroform solution yellowish green under the ultraviolet rays (The ray of wave-length 366 mμ was most effective for the emission).

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

References

1) Matuda, Tohoku J. Exp. Med., 1951, **53**, 333.

2) Masamune and Akama discovered first the in vitro conversion of Group B into Group A, and put forward a hypothetical mechanism of it in this Journal (1953, **57**, 310).

3) The substance was hydrolyzed with 1 *N* H_2SO_4 in a sealed tube for 4 hrs.

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Chemistry and Biology of Lipids

XVI. On Group Lipoid from Human Liver

Fourth Report*

By

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A batch of the group lipoid was separated in an electrophoretically homogeneous state from the liver of persons belonging to Group A, and after alkaline saponification, fractioned. The fraction, which was group active and will be referred to as "Partial Group Lipoid," was examined in detail regarding its composition. The nature of the remaining fragments was also investigated.

Experimental

1. Saponification of the group lipoid

The group lipoid was prepared according to the procedure preveously reported by Masamune, Maehara and Hakomori.[1a] It was a dark brown powder, electrophoretically homogeneous as described in the former report, and Group A active at a dilution of 1:3200. It was not inhibitory to isoagglutination of B erythrocytes even at a dilution of 1:100. 2.0 g. of the material were emulsified in 20 cc. of water and neutralized with 1 *N* NaOH, whereby the emulsion became transparent, and then an equal volume of 0.4 *N* NaOH was added. The dark brown solution given was placed in a thermostat of 20°C. After the lapse of 48 hours, it became markedly turbid and extremely ropy.

2. Fractionation

The viscid solution was freed from the precipitate by filtration through an asbestos layer in a glass filter. The bulk retained was dissolved in chloroform after thorough washing with 0.2 *N* alkali and distilled under reduced pressure, and the still-residue with yellow

* The most part was read before the 25th General Meeting of the Japanese Biochemical Society, Tokyo, April 26–28, 1953.[1]

silver luster was dried and set aside (Fr. I). The filtrate and washings above were combined and neutralized with 1 *N* H_2SO_4, then it was distilled in vacuo to one third the volume, by pouring little by little into a vacuous distillation-flask to avoid cumbersome foam formation. The condensate was acidified with 1 *N* H_2SO_4 to pH 1.2 under cooling with ice, and the precipitate thereby was centrifuged and washed twice with ice-cooled 0.1 *N* H_2SO_4—Ppt. I. The supernatant and washing were neutralized with 1 *N* NaOH and distilled in vacuo to dryness. Since the remainder here contained a considerable amount of sodium sulfate, it was extracted repeatedly with methanol (The insoluble non-colored part will be referred to as Fr. II). The methanolic solutions were also distilled to dryness—Fr. III. This fraction was fairly hygroscopic and tarry brown. Ppt. I above was exhausted several times with dry acetone. The acetone solutions were distilled to dryness (Fr. IV, a tarry clump). The acetone-insoluble part, dried in vacuo, was fractioned with methanol (Almost all the substance dissolved) and the solution was vacuum-distilled to dryness, washed with acetone and dried. A tan powder was given (Fr. V). The yield of the fractions were:

Fr. I, ca. 380 mg.
Fr. III, ca. 600 mg.
Fr. IV, ca. 700 mg.
Fr. V, 210 mg.

3. Isohemoagglutination-inhibiting potency of the fractions

TABLE I

Anti-isohemoagglutinative Potency of the Fractions after Alkaline Saponification of the Group Lipoid

A and B serum were both diluted to titre 16. −: no agglutination; ±: aggregates were seen when examined with the microscope; +: aggregates were seen with the naked eye; ++: marked aggl.; h: hemolysis.

Fraction	Group of red cells	Dilution of the fractions (1:)								
		50	10^3	2×10^3	4×10^3	8×10^3	1.6×10^4	3.2×10^4	6.4×10^4	∞
Fr. I	A	++	++	++	++	++	++			++
	B	++	++	++	++	++	++			++
Fr. II	A	h+	+	+	++	++	++			
	B	h+	+	+	+	+	+			
Fr. III	A		h+	+	++	++	++			
	B		h+	+	++	++	++			
Fr. IV	A		h	h±	+	++	++			
	B		h	h±	±	+	++			
Fr. V	A		h−	−	−	−	−	−	−	++
	B		h−	−	±	+	+	+	++	++

Our routine method was employed. Table I shows that only Fr. V was group active.

4. Chemical nature of Fr. I

It was dissolved in a smallest possible quantity of hot galcial acetic acid and slowly cooled. White silvery microcrystals precipitated. The deposit was filtered with suction and after washing with water, recrystallized from methanol and dried in air. Yield 150 mg. M. P.=65°C (sintered at 62°).

N (Lassaigne) and P (Plimmer) were absent. It gave a reddish purple colour with diazobenzenesulfonic acid and NaOH (Rosenthaler), and a purple colour with vanilline and sulfuric acid. But it did not reduce $AgNO_3$-NH_3 (Tollens) nor oxidize fuchsine-sulfurous acid (Schiff), nor formed a bromonitroso-compound with bromine and hydroxylamine (Piloty-Stock). Mol. wt. (Rast):

Substance 3.49 mg., campher 30.21 mg.; $\Delta t=15.3°$.

$$M=\frac{1000\times 3.49\times 40}{30.21\times 15.3}=302 \text{ (Calc. for } CH_3(CH_2)_{18}CH_2OH \text{ 328)}$$

Acetyl-derivative. 80 mg. of the substance were acetylated with acetic anhydride and anhydrous sodium acetate, and recrystallized from 70% alcohol, and the lustrous globular crystals were dried in a desiccator to a constant weight. Yield 50 mg. F. P. 40–43°C. Acetyl:

6.182 mg. substance, 0.01 *N* sodium thiosulfate used 1.72 cc.
$C_{20}H_{41}OCOCH_3$. Calc. 12.65%, found 11.96%.

Thus it appeared to be eicosanol-1.

5. Chemical nature of Fr. II

It was insoluble in most solvents excepting water, and ignition on a platinum plate gave rise to no appreciable carbonization. Furthermore it gave neither Molisch nor ninhydrin reaction. From these findings this fraction was suspected to be mostly inorganic salts particularly sodium sulfate.

6. Chemical nature of Fr. III

About 10 mg. of it were dissolved in 0.5 cc. of H_2O and a 0.05 cc. portion was immediately paper-chromatographed with Solv. I (water sat. *n*-butanol-water sat. phenol-ethanol (5:3:1)-NH_3) and 80% phenol-NH_3, but since the spots given were equivocal because of the salt effect, 60 mg. of the substance in 5 cc. of water were desalted together in a Consden, Gordon & Martin[2] apparatus (initial load: 150 V and 20 mA; duration: 5 hrs.). SO_4-ions, which had diffused from the anodic cellophane bag into the solution, were neutralized with baryta and the filtrate from $BaSO_4$ and washing were vacuum-distilled to dryness. The residue was dissolved in 1 cc. of water and 0.01–0.02 cc. portions were sampled for the chromatography series of Masamune and Hakomori[3]

consisting of five stages. Mostly 45 cm. long irrigation was effected in an ascending manner in a 3.5 cm. × 65 cm. tube at 24°±1°C, employing paper slivers (Toyo filter paper, No. 2) 60 cm. × 2.5 cm.

a) *First stage.* Technique:—i) Two paper slivers, on which the solution has been sampled, are irrigated one with Solv. I (water sat. *n*-butanol-water sat. phenol-ethanol (5:3:1)) in NH_3 atmosphere and the other with Solv. II (water sat. *n*-butanol-water sat. phenol-acetic acid (5:2:1)). Spot indication is carried out successively with various reagents. Namely, the slivers are at first sprayed with 0.5% iodine in 95% alcohol.[4] Choline and like N-methylated alkanolamines reveal themselves thereby as intensive reddish-purple spots, when excess iodine is fanned away. Secondary and primary amines react pale brown. [Rf of choline and its salts by Solv. I: free choline 0.63, sulfate and chloride 0.65; those by Solv. II: free choline 0.55, sulfate and chloride 0.54 (sulfate tails to 0.31).] After contouring the spots with pencil, the slivers are hung over an oven to remove the residual iodine. Secondly ninhydrin is applied to indicate ethanolamine, serine and hexosamines, if present. [Rf of ethanolamine and its salts by Solv. I: free ethanolamine 0.47 (tails to 0.42), hydrochloride and sulfate 0.48 (tail to 0.22); those by Solv. II: free ethanolamine 0.38, hydrochloride 0.22 (heads till the position of free ethanolamine). Rf of hexosamines by Solv. I: free hexosamines 0.15, hydrochlorides 0.10, sulfates 0.12; those by Solv. II: free hexosamines 0.17, hydrochlorides 0.10, sulfates 0.05. Rf of serine and glycine by Solv. I 0.06. Rf of glutamic and aspartic acid by Solv. I: these amino acids are scarcely moved.] The occurring spots are contoured here again. Thirdly, the slivers are immersed in 5% neutral lead acetate for a moment, and after washing exhaustively in running tap water for about 30 minutes, dired over an oven, followed by exposing to gaseous H_2S. The lead sulfide spot represents glycerophosphoric and/or inositolphosphoric acid. [Rf of Ba- and Na-salt of glycero- and inositolphosphoric acid by Solv. I: the salts are all scarcely moved.]

ii) Another pair of paper slivers, on which the substance has been developed as above, are first sprayed with aniline-hydrogen phthalate and afterwards with Dragendorf's reagent.[5] Non-amino and amino sugars react with the former reagent (Rf of galactose by Solv. I 0.10; that by Solv. II 0.15), and choline and like compounds with the latter.[6]

The chromatograms reproduced in Figs. 1A and 1B disclosed the absence in Fr. I of choline, ethanolamine and sugars and the presence of amino acids and a phosphate or phosphates.

b) *Second stage.* Usual one- and two-dimensional amino acid chromatographies (The former descending) were adapted, employing 80% phenol (in atmosphere of NH_3) and *n*-butanol-acetic acid-water (4:1:2) as solvents.

The chromatograms given (Figs. 2A and 2B) showed spots corresponding to aspartic and glutamic acid, glycine, alanine and arginine.

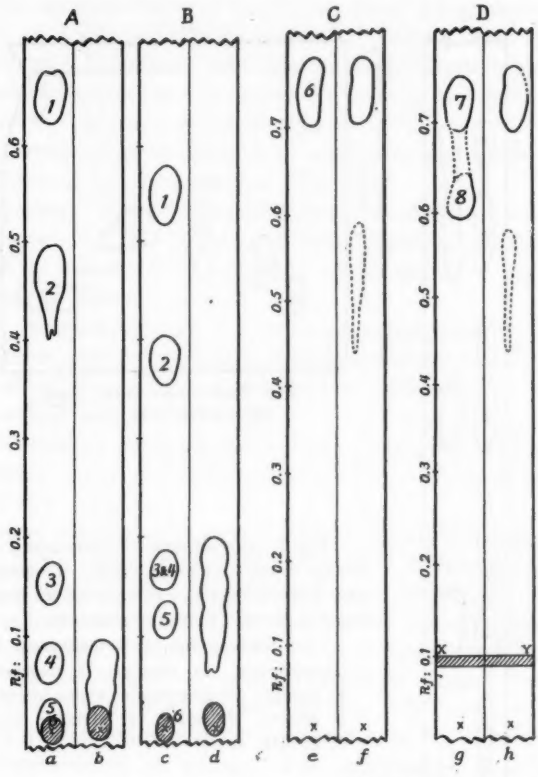

Fig. 1. First (A and B), Fourth (C) and Fifth (D) stage chromatograms of Fr. III of the liver group lipoid, irrigated (A) with Solv. I in an atmosphere of NH_3, (B) with Solv. II and (C and D) with Solv. VI. Temperature of test 24°±1°C. Solvent fronts run 45 cm. in (A) and (B) and 30 cm. in (C) and (D). See the text regarding details.

a, c, e, g: reference runs. b, d, f, h: main runs. 1: choline, 2: ethanolamine, 3: glucosamine hydrochloride, 4: glycine, 5: aspartic acid, 6: Ba-glycerophosphate (α- + β-), 7: Ba-α-glycerophosphate-$Ba(NO_3)_2$ complex, 8: Ba-β-glycerophosphate-$Ba(NO_3)_2$ complex, X—Y: $Ba(NO_3)_2$-band.

Alanine preponderated among the others.

c) *Third stage.* The presence of sugars was already disproved by the first stage chromatogram. It was ascertained here by Masamune & Maki technique, taking twice the amount of the material in other runs, that is, 0.04 cc. of the solution.

d) *Fourth stage.* To precisely identify the lead spots in the first

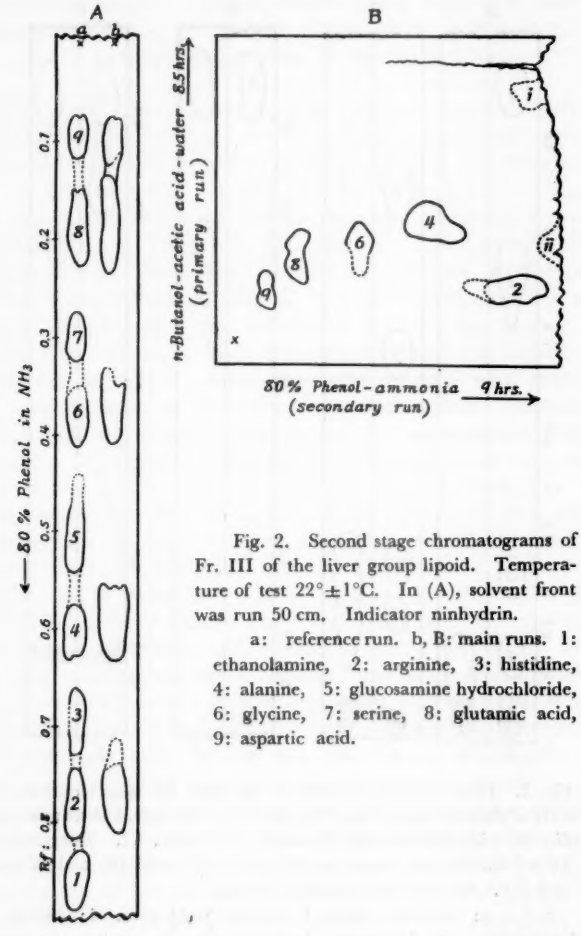

Fig. 2. Second stage chromatograms of Fr. III of the liver group lipoid. Temperature of test 22°±1°C. In (A), solvent front was run 50 cm. Indicator ninhydrin.

a: reference run. b, B: main runs. 1: ethanolamine, 2: arginine, 3: histidine, 4: alanine, 5: glucosamine hydrochloride, 6: glycine, 7: serine, 8: glutamic acid, 9: aspartic acid.

stage chromatograms, the material was chromatographed, using 80% phenol-acetic acid-water (1:1:1) (Solv. VI) for irrigation. Lead spots corresponding to Ba-glycero- and Ba-inositol-phosphate [approximate Rf of Ba-glycerophosphate and Ba-inositol-phosphate by Solv. VI 0.75 and 0.55 respectively; those of free glycero- and inositol-phosphoric acid: 0.37–0.5 and 0.13–0.27 respectively by Solv. IV; 0.80 and 0.60 respectively by Solv. V] were given. The spot of Ba-inositolphosphate was very weak. See Fig. 1C.

e) *Fifth stage.* The substance was run on a paper sliver with a

horizontal 0.5 cm.-wide band of $Ba(NO_3)_2$, ruled 3 cm. above the bottom with 0.05 cc. of a 5% solution of this salt. Solvent used VI. Solvent front run 30 cm. Barium-α- and barium-β-glycerophosphate were run simultaneously for reference (The mean Rf value of the complex of α-salt, 0.73; that of the complex of β-salt, 0.64).

Spots corresponding to Ba-salts of α-glycero- and inositol-phosphoric acid were detected in the main run (Fig. 1D).

f) *Addendum.* When a chromatogram was irrigated with *n*-butanol-acetic acid-water (4:1:2), two brown spots appeared at the positions of approximate Rf values of 0.3 and 0.7, which gathered together at the front by either of Solvs. I and II.

In short, definitely identified were aspartic and glutamic acid, glycine, alanine, arginine and inositol- and α-glycero-phosphoric acid in Fr. III. Two unidentified components were found in addition.

7. Chemical nature of Fr. IV

It was soluble in most organic solvents. It dissolved in alkali also, but the solution changed turbid when acidified. Carboxyl was demonstrated by Feigle reagent.

An alkaline solution of it was acidified with H_2SO_4 and shaken with portions of ether. Most of the colored matter remained in the watery layer. The yellowish ethereal solutions were combined, washed with H_2O and distilled to dryness. The still-residue was dissolved in a little hot 50% ethanol and cooled slowly at room temperature, followed by standing in an ice chest. A white crystalline deposit occurring was filtered off and dried in vacuo. Yield 80 mg. M. P.=62–63°C. Mixed melting point with palmitic acid 63° (sintered at 61°). The brown watery layer was concentrated in vacuo. The still-residue here showed the positive Rosenthaler (for alcohol) and Feigle[5] (for carboxyl) test, suggesting a hydroxy-fatty acid.

8. Chemical nature of Fr. V (Partial Group Lipoid)

It was dried in vacuo over H_2SO_4 to a constant weight. Only one boundary was demonstrated after electrophoresis, irrespective of the pH of the buffer solution employed (Fig. 3). The boundary moved in the buffer of pH 7.8 more quickly than that of the original group lipoid in the same buffer solution. Analysis: N (micro Kjeldahl) 3.2% (Prep. I, sample obtained above), 3.5% (Prep. II, sample after another run[6a]); P (Haneda, Sinokawa & Yasuoka's modification of Plimmer method) 1.8% (Prep. I), 2.1% (Prep. II); total reduction as glucose (Masamune & Tanabe)[6b] 31.1% (Prep. II); ash (Pregl method without use of H_2SO_4) 3.3% (Prep. I), 8.8% (Prep. II).

A) The water-insoluble components

When the Partial Group Lipoid was heated with a dilute watery

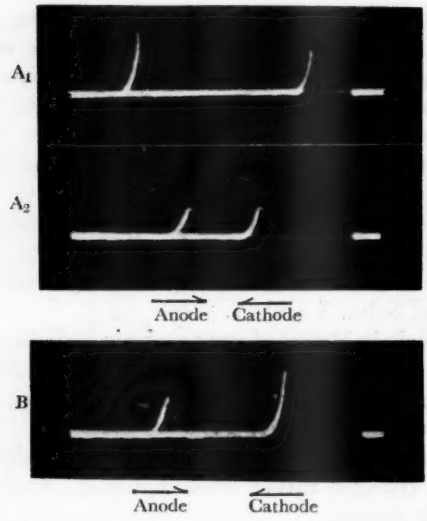

Fig. 3. Electrophoretic patterns of 1% solutions of the Partial Group Lipoid in (A) phosphate buffer of pH 7.8 and I 0.29 and (B) acetate buffer of pH 4.6 and I 0.10. Temperature of test 20°C. Exposure after starting current 20 and 40 minutes respectively in (A_1) and (A_2) and 15 minutes in (B). Mobility:

$$(A_2),\quad \frac{\frac{1.35+1.45}{2}\times 0.0102\times 0.3}{0.01\times 2400} = 17.8\ \mathrm{cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot 10^5}$$

$$(B),\quad \frac{\frac{1.15+1.20}{2}\times 0.0041\times 0.3}{0.01\times 900} = 16.1\quad ,,$$

mineral acid solution, a dark resinous substance formed abundantly. Hence, methanolysis in benzene was tried.

100 mg. of the substance were dissolved in 30 cc. of benzene, and after addition of 10.5 cc. of the HCl-saturated methanol, stood in a sealed tube at 50°C for 18 hours, when the mixture turned dark red. On cooling it in water, some deposit appeared, which was centrifuged off (Ppt. a). The supernatant was distilled in vacuo, supplementing benzene from time to time, to expel off most of HCl and then shaken with changes of water. The washings (Fl. a) were set aside. The benzene solution, which had changed extremely cloudy, was centrifuged. The centrifugate (Ppt. b) amounted to 68 mg., when dried in a desiccator. The supernatant was distilled in vacuo to dryness and the still-residue was put together with Ppt. b.

The combined substances (Div. a) behaved towards various solvents

TABLE II

Solubilities of Div. a in 8.,A)

−: insoluble, ±: faintly sol., +: pretty sol., ++: very sol.

Solvent	Water			Methanol	Ethanol	Butanol	Amyl-alcohol	Acetone	Ether	Benzene
	plain	alkaline	acidic							
Solubility	±	±	−	++	+	+	++	++	±	±

as is shown in Table II. 0.05 cc. portions of its 1% solution in methanol were sampled on several slivers to subject to one of the First stage chromatographies of Masamune and Hakomori.[3] Solvent: water sat. *n*-butanol-water sat. phenol-ethanol (5:2:1.5) (Solv. III). Solvent front run 45 cm. Each paper sliver was sprayed successively with ninhydrin, alkaline methylred, $AgNO_3$-NH_3 and Schiff reagent. The first two reagents ninhydrin and alkaline permanganate develop a spot of sphingosine at the position of Rf 0.80 (Rf of sphingosine sulfate 0.77), the last two reagents $AgNO_3$-NH_3 and Schiff reagent plasmals (An elongated spot of Rf 0.65–0.75 occurs, if plasmals are present). In the present case, neither a spot of sphingosine nor that of fatty aldehydes was revealed. But, since an acidic spot was recognized by alkaline methylred in the solvent front region, this part was cut off from three unsprayed slivers and extracted with ether. The ethereal solution gave apparently positive carboxyl-reaction of Feigle.[7] Moreover, at a position of Rf 0.85 accumulated the brown components into a brown spot which did not react with any of the indicators above excepting alkaline permanganate. Next, 30 mg. of the material Div. a were distilled in an Eder-Werner-Klein apparatus under high vacuum. A tiny portion was sublimed at 80°C and 0.03 mm. Hg, leaving the greater part at the bottom.

The results above suggest, we think, that the water-insoluble methanolysis-products comprise little of simple fatty acids or their esters, even though it looked to be chiefly composed of carboxylic acids or their esters (S. below).

The water insoluble was next fractioned by liquid adsorption chromatography:—A 15 cm. high alumina[8] column was prepared according to the routine wet method by means of acetone, and an acetone solution containing 50 mg. of the substance was poured onto the column, whereby a colored band formed at the top of the column. Elution was processed with 25 cc. of acetone first, secondly with 35 cc. of abs. methanol, thirdly with 35 cc. of 80% methanol, and lastly with 150 cc. of a methanol-

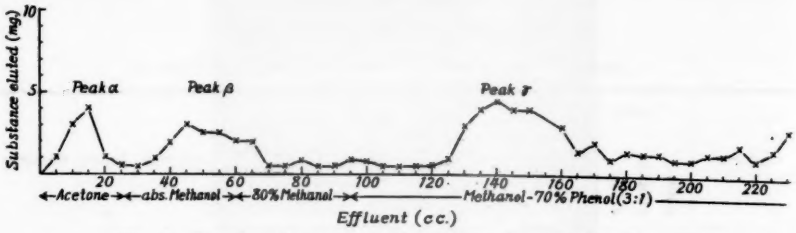

Fig. 4. Showing the amounts of substance in successive 5 cc. eluate portions of the water-insoluble components of the Partial Group Lipoid adsorbed on alumina.

TABLE III

Reactions of the Divisions obtained after Liquid Adsorption Chromatography of the Water-insoluble Part of the Partial Group Lipoid

−: no reaction, ±: faint r., +: moderate r., ++: marked r.

Reaction	Div. α	Div. β	Div. γ
Ninhydrin*	−	−	−
Alkaline-permanganate*	±	±	++
Bromine (addition)	−	−	+
Ammoniacal-silver nitrate*	−	−	±
Fuchsine-sulfurous acid*	−	−	−
Hydroxamic acid-$FeCl_3$ (after Feigle)†[7]	+	+	+
Diazobenzenesulfonic acid-NaOH (Rosenthaler)	−	−	++
Nitroprusside			
with acetone‡	−	−	−
with aldehyde§	−	−	−
Dragendorf			
direct//	−	−	−
after treatment with dimethylsulfate¶	−	−	−

* Spot test. † Processed without prior treatment with $SOCl_2$. ‡ Sphingosine gives a purple colour when its acetone solution is mixed with Na-nitroprusside (The reaction occurs slowly as compared with ethanolamine). § N-methylsphingosine gives a blue colour with Na-nitroprusside and acetaldehyde (The reaction occurs slowly as compared with diethylamine). // N-methylsphingosine gives a yellowish orange ppt. ¶ Sphingosine gives a yellowish orange ppt.

70% phenol (3:1) mixture. Successive 5 cc. portions of the effluent were separately collected, evaporated on a steam bath and dried in a desiccator. The amounts of the eluate portions are plotted in Fig. 4. The curve shows three peaks. In Table III are embodied the consequences of the test-tube and spot tests of the divisions corresponding to the peaks (mountains),

wherefrom the Peak α and Peak β substances (Divs. α and β) are thought to have been the substances sublimed when distilled under very low pressure, and the Peak γ substance (Div. γ) unsaturated polyhydroxy acids or their esters. And judging from its melting point (27–35°), Div. α was most probably a mixture of methylpalmitate and methylstearate. None of the Divs. gave the positive test for free or N-methylated amino group, so that the presence of sphingosine in Fr. V was not accepted. Plasmals, if present, would have been converted into acetal during methanolysis. Therefore, a portion of the Div. γ was hydrolyzed by heating with 0.1 *N* HCl under reflux for 30 minutes to cleave the possible acetals. The hydrolysate was neutralized and evaporated to dryness on a water-bath and the residue taken up in methanol. The solution, however, gave rise to no coloration of Schiff fuchsine reagent. On the other hand, the Partial Group Lipoid itself gave negative Feulgen test on paper.

B) The water-soluble components

Qualitative examination

The small precipitate, which had occurred on cooling the benzene solution after methanolysis, (Ppt. a) was dissolved in the washings of the solution (Fl. a) and after addition of water to 20 cc. and 0.2 *N* HCl to 0.1 *N*, heated under reflux in a boiling water-bath for 1 hour to hydrolyze the methylesters and methylglycosides. To the hydrolysate was added a hot Ag_2SO_4 solution until no more precipitate occurred. The precipitate (Ppt. *a*) was filtered off, and put away for the examination below. The filtrate and washings were treated with H_2S and the subsequent filtrate with baryta (Baryta was added to a pH of about 6.6). The precipitates Ag_2S and $BaSO_4$ were washed energetically with water, and the mother fluid and washings were distilled in vacuo to about 1 cc., of which 0.01 cc. portions were paper-chromatographed by the technique described in connection with Fr. III.

a) The first stage runs carried out with Solvs. I and II (Figs. 5A and 5B). Ethanolamine, hexosamine, serine and/or glycine, glutamic and/or aspartic acid and probable glycerophosphoric acid were demonstrated, but neither choline nor betaine.

b) By the second stage runs (one-dimensional 80% phenol-NH_3 chromatography and two-dimensional *n*-butanol-acetic acid-water (4:1:2) /80% phenol-NH_3 and water sat. collidine-diethylamine/80% phenol-NH_3 chromatographies (Fig. 6)), glycine, serine and glutamic acid were identified.

c) By the third stage runs (*n*-butylacetate-acetic acid-ethanol-water (3:2:1:1)[9] and amylalcohol-pyridine-water (7:7:6) (Werner-Odin) chromatographies) were demonstrated galactose, mannose and chondros-

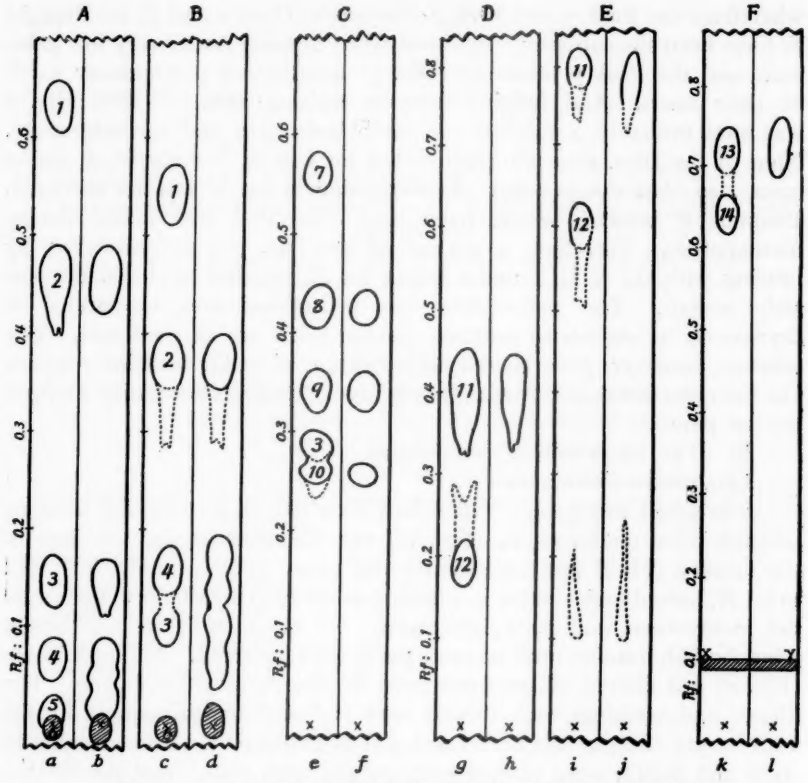

Fig. 5. First (A and B), Third (C), Fourth (D and E) and Fifth (F) stage chromatograms of the water-soluble part of Fr. V (Partial Group Lipoid) irrigated with (A) Solv. I-NH_3, (B) Solv. II, (C) amylalcohol-pyridine-water (7:7:6) of Werner & Odin, (D) Solv. IV, (E) Solv. V and (F) Solv. VI. Temperature of test 24°±1°C. Solvent fronts run 45 cm. in (A)–(C) and 30 cm. in (D)–(F).

a, c, e, g, i, k: reference runs (g and i: a hydrolysate of crude inositolphospholipid fraction (Folch's I. P. Fr. 1[8a]) of brain cephalin was developed). b, d, f, h, j, l: main runs. 1: choline, 2: ethanolamine, 3: glucosamine hydrochloride, 4: glycine, 5: glutamic acid, 6: Na-glycerophosphate, 7: rhamnose, 8: mannose, 9: galactose, 10: chondrosamine hydrochloride, 11: glycerophosphoric acid (α- + β-), 12: inositolphosphoric acid, 13: Ba-α-glycerophosphate-$Ba(NO_3)_2$ complex, 14: Ba-β-glycerophosphate-$Ba(NO_3)_2$ complex, X—Y: $Ba(NO_3)_2$-band.

amine, but neither fucose nor glucosamine (Fig. 5C). Presence of fucose was further denied by the spectrophotometric thionalide-HCl analysis of

Masamune & Ogawa.[10]

d) The precipitate, that had occurred on addition of Ag_2SO_4 to the hydrolysate, (Ppt. *a*) was decomposed with hydrogen sulfide, and after filtering off of Ag_2S, the filtrate was subjected to repeated vacuum distillations, redissolving the residue with water in the second and following times, to eliminate HCl as far as possible. The final still-residue was taken up in a small quantity of water and its portions were examined by paper partition chromatography with 80% ethanol (Solv. IV) and ethanol-methanol-glycerol-water (2:1:1:2) (Solv. V). Indicator, lead acetate-H_2S. Spots of free glycerophosphoric acid were shown (A hydrolysate of crude inositolphospholipid fraction of brain cephalin was run for reference (Figs. 5D & 5E).

e) The pH of the balance of the solution was carefully adjusted to 7.8 with baryta in order to change free glycerophosphoric acid into barium salt. The solution was then evaporated on a boiling water-bath nearly to dryness and five drops of water added. And a 0.03 cc. portion of the solution was sampled to gain a Fifth stage chromatogram (Solv. VI) as in 6,e). The spot given corresponded to the position of Ba-*α*-glycerophosphate-$Ba(NO_3)_2$ complex (Fig. 5F).

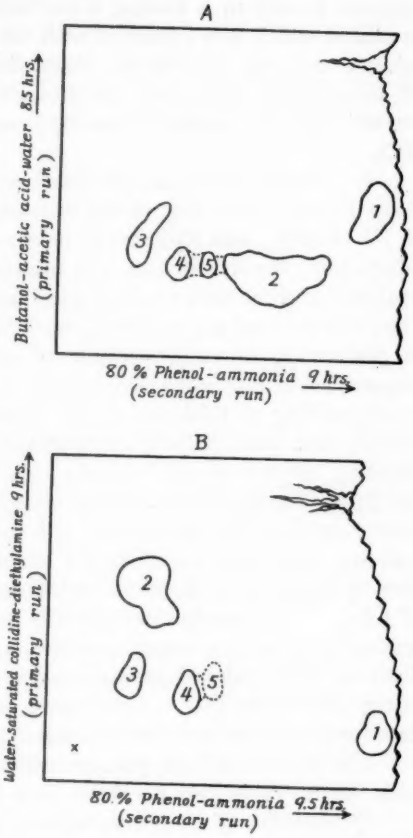

Fig. 6. Second stage two-dimensional chromatograms of the water-soluble part of Fr. V (Partial Group Lipoid). Temperature of test 22°±1°C. Indicator ninhydrin.
1: ethanolamine, 2: glucosamine hydrochloride, 3: glutamic acid, 4: serine, 5: glycine.

Additional experiments

a) *Absence of neuraminic acid.* About 5 mg. of the Partial Group Lipoid were dissolved in 1 cc. of water and after addition of 1 cc. of Bial

reagent, heated in a boiling water-bath for 5 minutes. A red dye was produced which was extracted with amylalcohol. But, the amylalcoholic solution did not exhibit the absorption at 570–600 mμ[11] characteristic of neuraminic acid and, furthermore, a resembling amylalcohol-extractible dye was produced also by heating the substance with plain conc. HCl.

b) *Absence of inorganic phosphoric acid in an acid hydrolysate of the Partial Group Lipoid.* 48.88 mg. of the substance were finely suspended in 15 cc. of 1 *N* H_2SO_4, and hydrolyzed in a sealed tube for 4 hours at 100°C. When cold, the hydrolysate was filtered in a filtros with mild suction repeatedly, and the bulk retained was washed well with water and set aside. (The filtrate and the washings were collected separately.) The filtrate (a portion) gave no precipitate of ammonium-phosphomolybdate with ammonium nitrate and Pregl reagent on heating. Next 2 cc. of the filtrate corresponding to 6.52 mg. of the substance was charred up with conc. H_2SO_4 and conc. HNO_3 to estimate organic phosphorus according to Haneda, Sinokawa and Yasuoka. It was found to amount to 1.75% of the Partial Group Lipoid. Finally the balance of the filtrate and washings were combined and exhausted with ether (in a Sudo & Kumakawa apparatus), and after expelling off of ether by aeration, freed from SO_4-ions by means of a slight excess of the sat. baryta, followed by passing of CO_2. The precipitates $BaSO_4$ and $BaCO_3$ were centrifuged and washed with boiling water, and the supernatant fluid and washings were distilled under reduced pressure to a small volume (about 0.5 cc.). Drops of the condensate (Fl. *a*) were tested on a filter paper by Feigle's molybdate-benzidine method for inorganic phosphates with negative result.

The water-insoluble part set aside above (35.12 mg., 72%) contained only 0.08% N (micro Kjeldahl) and 0.20% P.

c) *Isolation of Ba-glycerophosphate from the acid hydrolysate in b*). To the balance of Fl. *a* above, 5 cc. of ethanol were added, and the mixture was stood overnight in an ice chest. A white deposit, that had occurred, was filtered and dried in vacuo over $CaCl_2$. Yield 9 mg. It was taken up in 5 cc. of water. The insoluble part was rejected by centrifugation and the clear supernatant was precipitated with the sat. lead acetate, centrifuged and washed well with water. This lead salt was decomposed in water with hydrogen sulfide and the centrifuged supernatant was neutralized with baryta, distilled in vacuo and precipitated with alcohol. The barium salt here was dissolved in 0.5 cc. of H_2O, reprecipitated once with 8 cc. of ethanol and dried in vacuo over H_2SO_4 to constant. Yield 4 mg. Molisch negative. Ash($BaSO_4$) (Pregl):

0.645 mg. substance, 0.862 mg. ash.
$C_3H_7O_6PBa$. Calc. 75.9%; found 74.8%.

TABLE IV

Composition of the Water-soluble Part of the Partial Group Lipoid

Analysis	In per cent of the Partial Group Lipoid		In equivalents per equivalent weight	
	Prep. I	Prep. II	Prep. I	Prep. II
Chondrosamine*	9.0	8.8	2.1	1.8
Galactose†	15.0	15.3	3.2	3.0
Mannose†	8.5	8.4	2.0	1.7
Glutamic acid*	8.1	7.1	2.2	1.7
Serine*	2.2	2.1	0.8	0.7
Glycine*	1.9	1.8	1.2	1.0
Ethanolamine*	3.0	1.7	2.0	1.0
Glycerophosphoric acid‡	9.9	11.5	2.3	2.4

* A modification of Awapara & Landua method[12] with a modified Stein & Moore's ninhydrin reagent[13] (See text). † Masamune & Ogawa method. ‡ Glycerophosphoric acid was calculated from the P content of the Partial Group Lipoid, because neither inorganic nor inositol-phosphoric acid was found in the acid hydrolysate of the substance.

Quantitative analysis

The figures obtained are embodied in Table IV. The amino acids and amines were determined by a following technique:—

An accurate amount (5–10 mg.) of the substance is weighed in a small tube (0.5 cm. diameter, 1.5 cm. hight) and hydrolyzed with 6 *N* HCl at 100°C for 4 hours under a small returning condensor (15 cm. hight) fixed by a ground joint. After hydrolysis, the tubelet is stood in vacuo over NaOH to eliminate HCl (Caution not to allow too much foam-formation!). The evaporated residue of the hydrolysate is dissolved in 0.1 cc. of water, slightly alkalized with 0.2 *N* NaOH to pH 7.5–8.0, and filled up to a definite quantity. The humin and others insoluble are centrifuged off. An aliquot of the supernatant is chromatographed on a paper sliver with 80% phenol without use of ammonia. After the development (The solvent front is run 50 cm. from the starting point), the chromatogram is nearly freed from phenol by washing with ether and dried over an oven, followed by spraying with 0.01% ninhydrin. The spots indicated are cut off separately. Each of the pieces is finely sheared, covered under 1.5 cc. of a modified ninhydrin reagent of Stein & Moore[13] (a 1% ninhydrin solution in a mixture of ethylcellosolve and 0.2 *M* citrate buffer of pH 5.0 containing 0.16% $SnCl_2$ in the proportion of 1:1 by volume) in a narrow vessel (hight 8 cm., extents sidewise 0.5 and 2.0 cm.) and heated, as loosely capped, for 20 minutes in a vigorously boiling water-bath. Cooled. The colored solutions are transferred separately to a 25 cc. volumetric flask through a small filter. The stains on the paper fibers are washed out with changes of 50% ethanol and the washings are also transferred to the respective flasks, followed by filling up to the mark

with 50% ethanol. The color depths are immediately estimated at 570 mμ in a spectrophotometer against the blank.

Judging from the table, the water-soluble of the Partial Group Lipoid were made up of α-glycerophosphoric acid, ethanolamine, glutamic acid, serine, glycine, galactose, mannose and chondrosamine in molecular proportions of 2:1–2 (different according to the preparation):2:1:1:3:2:2.

Summary

The group lipoid separated from Group A human livers in a practically pure state was saponified with 0.2 *N* NaOH into four fractions. One of the fractions was eicosanol-1 and a second a mixture of several amino acids and α-glycero- and inositol-phosphoric acid, a third proved to contain palmitic acid, and a fourth was a " Partial Group Lipoid " that proved homogeneous after electrophoresis. Furthermore it was shown that the Partial Group Lipoid was made up of α-glycerophosphoric acid, ethanolamine, glutamic acid, serine, glycine, galactose, mannose, chondrosamine in molecular proportions of 2:1–2:2:1:1:3:2:2 and carboxylic acids.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us, which is gratefully acknowledged. H. Masamune.

References and Notes

1) Masamune, Maehara, Hakomori & Hirata, Tohoku J. Exp. Med., 1953, **58**, 216.
1a) Masamune, Maehara & Hakomori, *ibid.*, this volume p. 225.
2) Consden, Gordon & Martin, Biochem. J., 1947, **41**, 591.
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4) Brante, Nature, 1949, **163**, 651.
5) The reagent was modified for the use in alkaloid paper chromatography by Hashimoto (Nature, 1951, **168**, 139), but the one employed by us was merely a 5-fold diluted solution of the original (Cited in Winterstein & Trier, Die Alkaloide, Gebrüder Borntraeger Berlin, 1931 p. 965).
6) The iodine method has an advantage due to the reversible nature of the reaction, but it is not specific because even such cations as Na^{+}, K^{+} and Ba^{++} react with iodine. It is, therefore, recommended to scrutinize the result with Dragendorf reagent.
6a) It possessed similar anti-isohemoagglutinative potencies as Prep. I.
6b) First method was adapted without use of phosphotungstic acid; hydrolysis 4 hours.
7) Feigle, Qualitative Analyse mit Hilfe von Tüpfelreaktionen, Akademische Verlagsgesellschaft Leipzig, 1938, p. 400.
8) Alumina (Takeda) was activated by ignition in a nickel dish over a free flame for 1 hour with following 30 minutes' washing under running water.
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Chemistry and Biology of Lipids

XVII. Composition of the Group Lipoid of Pig Lung

By

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The group lipoid from pig lungs[1] was cleft with alkali and the fractions obtained were investigated regarding their components.

Experimental

Alkaline Cleavage and Fractionation

1 g. of the group lipoid prepared as already described[1] was dissolved in 10 cc. of water (A clear brown solution was given) and after addition of an equal volume of 0.4 *N* NaOH, stood for 3 days at 20°C, whereby a voluminous, glistening, white precipitate appeared. The mixture was then diluted with 130 cc. of water and filtered with suction. The bulk on the funnel was washed with 10 cc. of 0.2 *N* NaOH and changes of water and dried in a desiccator—Fr. I. Crop, 110 mg. To the filtrate were added about 18 cc. of 1 *N* H_2SO_4 in small portions, whereby the solution was changed more acid than pH 1.2 (towards thymol blue) and a brown flocculent precipitate occurred. Since the precipitate could not be easily separated, the solution containing it was immediately exhausted with three 60 cc. portions of ether in a separatory funnel. The ethereal solutions were combined, dried with anhydrous sodium sulfate and distilled in vacuo. And the dark brown sticky still-residue was kept under 50 cc. of dry acetone overnight, washed with 60 cc. portions of the acetone and placed in a desiccator—Fr. II. It weighed 126 mg. The brown-colored washings (acetone solutions) were also distilled and the dark brown, sticky remainder was dried in a desiccator—Fr. III. The watery layer after ether extraction above was neutralized with 1 *N* NaOH, distilled under diminished pressure to dryness and extracted with three 40 cc. portions of methanol of 40°C and with cold methanol. The yellowish brown methanolic solutions were distilled to dryness, washed with acetone and dried, as had been the ethereal solution above. A slightly brown powder weighing 580 mg. was given—Fr. IV. The non-colored

TABLE I

Occurrence of Agglutination of A and B Erythrocytes by Normal Sera treated with the Pig Group Lipoid and its Franctions

The assay was processed by our usual procedure. A and B sera had agglutinin titres of 160 and of 320 and were diluted 10- and 20-fold respectively. All the substances excepting Fr. I gave a solution in physiol. saline with or without the aid of NaOH. Fr. I was ground into a fine powder and suspended in physiol. saline and progressively diluted. −: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., ###: strong aggl., h=hemolysis.

Substance	Group of red cells	Dilution of substances (1:)									
		10^2	2×10^2	3×10^2	4×10^2	5×10^2	10^3	2×10^3	3×10^3	4×10^3	∞
Group lipoid	A	+	+	+	+	++	###	###		###	###
	B	−	−	−	−	−	−	−		+	###
Fr. I	A	++	++		###		###	###	###		
	B	++	###		###		###	###	###		
Fr. II	A	++	###		###		###	###	###		
	B	−	−		−		−	−	+		
Fr. III	A	h	h		h		h	h	h		
	B	h	h		h		h	h	h		
Fr. IV	A	++	###		###		###	###	###		
	B	++	++		###		###	###	###		
Partial Group Lipoid	A		+	+	+	+	++	++	++	++	
	B		−	−	−	−	−	−	−	+	

methanol-insoluble residue was not charred by heating over a flame, but was left as an ash. All the fractions, Fr. II excluded, showed neither A nor B blood group activitity as is illustrated in Table I.

Batches of the fractions were prepared by repetition of the procedure.

Purification of the Fractions and Identification of the Components

Fr. I. 100 mg. of it were dissolved in 20 cc. of 80% acetic acid by warming in a water-bath of 70°C and the clear brown solution was stood in the bath, allowed to cool gradually by turning off of the gas, and, when cold, further in an ice chest overnight. The crystalline deposit was filtered off and washed with cold 80% acetic acid and with water in turn. 86 mg. was the yield. It was further recrystallized twice to a constant melting point. The amount of substance was reduced to 76 mg. Some amount of crystals was obtained from the mother fluids and 80% acetic acid-washings by precipitation with water and crystallization in a manner similar to above. And from the mother fluid

here was recovered by distillation in vacuo a light-brown non-sticky powder weighing 30 mg., which was soluble neither in organic solvents nor in dilute hydrochloric acid and nearly all remained as an ash on ignition.

The crystalline product sintered at 72°C and melted at 74°C in coincidence with tetracosylalcohol. It was soluble in ethanol and chloroform, and free from N and P. It was also devoid of aldehyde- (Schiff and Tollens tests) and ketone-group (Piloty-Stock and bromonitroso reactions), but gave diazobenzenesulfonic acid- and vanilline-reaction for alcohol. Molecular weight (Rast):

$$M=\frac{1000\times 3.177\times 40}{32.53\times 11}=355 \text{ (Calc. for } CH_3(CH_2)_{22}CH_2OH, 354).$$

Acetylderivative: This was prepared by means of acetic anhydride and anhydrous sodium acetate and purified from a mixture of ethanol and water (1:4 by volume). The product melted at 56.5°C, having sintered at 55.5° (tetracosanolacetate 57°). CH_3CO (Suzuki's modification of Friedrich, Rapoport and Sternberg method[2]):

7.396 mg. substance; 0.01 *N* Na_2SO_3 used, 1.86 cc.
$C_{24}H_{49}OCOCH_3$. Found 10.81%; calc. 10.86%.

Fr. II. This fraction was purified, taking the blood group potency as an indication, as follows:—120 mg. of it were extracted exhaustively with 30 cc. portions of 95% methanol and the solutions were distilled together in vacuo to dryness. The residue was further washed with acetone and dried, and 80 mg. of a substance was given. This was again dissolved in 100 cc. of 95% methanol and about 20 mg. of $BaCl_2{\cdot}2H_2O$ were added to carry down all the precipitable. And, the centrifuged supernatant was distilled to dryness under reduced pressure. The still-residue here was taken up in 50 cc. of chloroform and after rejecting the insoluble part, distilled to dryness, followed by washing with acetone and drying in vacuo over $CaCl_2$. A light-brown powder was obtained. 32 mg. was the crop of it. It inhibited the isoagglutination of B erythrocytes

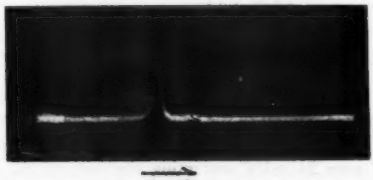

Fig. 1. Showing the electrophoretic pattern of a 1% solution of the Partial Group Lipoid in carbonate buffer, pH 8.9, I=0.105; temperature 15°C; current 10 mA; exposure after starting current 20 minutes. Ascending limb only is shown.

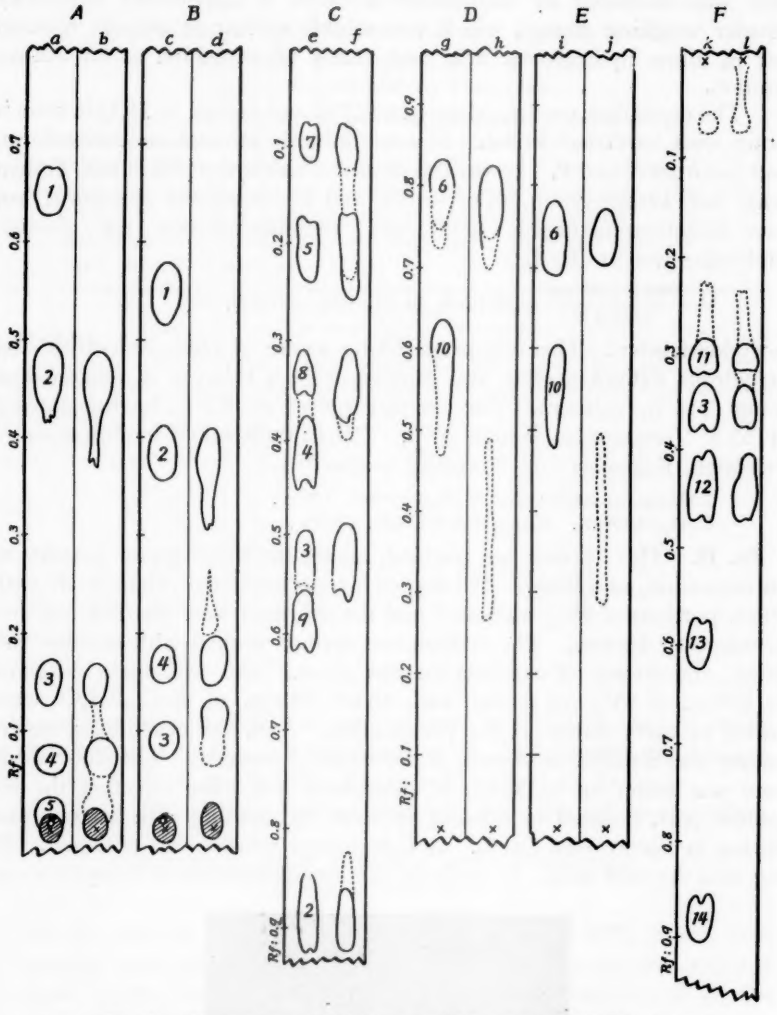

Fig. 2. Chromatograms of the water-soluble components in (A—E) a 6 *N* HCl- and (F) a 1 *N* H_2SO_4-hydrolysate of the Partial Group Lipoid. Temperature of tests 24°±1°C excepting the case of (C) where irrigation was effected at 22°C. (A) and (B): First stage chromatograms irrigated (A) with water sat. *n*-butanol-water sat. phenol-ethanol (5:3:1) (Solv. I) in an atmosphere of NH_3 and (B) with water sat. *n*-butanol-water sat. phenol-gl. acetic acid (5:2:1) (Solv. II) in the ascending manner. Solvent fronts run 45 cm. Spots indicated successively with iodine, ninhydrin and lead

at a dilution of 1:3000 (See Table I). The balance of the product (22 mg.) was freed from barium by washing with 1 cc. portions of 0.1 *N* HCl and after further washing with some water and changes of acetone, dried. 18 mg. of a powder with yellowish hue were given—" Partial Group Lipoid." This substance was perfectly soluble in benzene and as group potent as above. From 3 g. of the lung group lipoid were thus obtained 86 mg. of the Partial Group Lipoid in all. The products united showed following properties:—

Electrophoresis. It was shown to be essentially homogeneous after electrophoresis (Fig. 1).

Solubility. It was readily soluble in benzene, chloroform and plain water, but insoluble in abs. methanol, abs. alcohol and acetone as well as in water acidulated to pH 1.2.

Test-tube tests. Molisch ±, Lassaigne for N +, P ±, the indirect Osaki-Turumi −, Elson-Morgan for hexosamine in the hydrolysate +.

Paper partition chromatography (The writer is indebted to Mr. S. Hakomori). Aspartic and glutamic acid, serine, ethanolamine, chondrosamine, glucosamine, galactose and glycerophosphoric acid were demonstrated as water-soluble components by the procedure of Masamune and Hakomori[3]:—1. 20 mg. of the Partial Group Lipoid were heated with 2 cc. of 6 *N* HCl under reflux at 100°C for 5 hours. The resinous deposit was filtered off and washed thoroughly with water. The filtrate and washings were combined and HCl was expelled off as much as possible by standing the solution in vacuo over NaOH, adding water from time to time, then, the residue was dissolved in water, adjusted to pH 7.5 with NaOH (0.2 *N*) and vacuum-distilled to dryness. The remainder hereby was taken up in 0.3 cc. of water and 0.05 cc. portions were sampled separately on paper strips

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acetate-H_2S. (C): Second stage chromatogram, irrigated with 80% phenol in an atmosphere of NH_3 in the descending manner. Solvent front run 50 cm. Indicator ninhydrin. (D) and (E): Fourth stage chromatograms, irrigated with (D) ethanol-methanol-glycerol-water (2:1:1:2) (Solv. V) and (E) 80% phenol-gl. acetic acid-water (1:1:1) (Solv. VI) in the ascending manner. Solvent fronts run 30 cm. Indicator lead acetate-H_2S. (F): Third stage chromatogram, irrigated with *n*-butanol-pyridine-water (5:3:2) in the descending manner. Duration of test 24 hrs. Indicator aniline hydrogen phthalate.

a, c, e, g, i, k: reference runs (g–i processed with a hydrolysate of a crude inositolphospholipid fraction of brain cephalin of Folch[10]). b, d, f, h, j, l: main runs. 1: choline, 2: ethanolamine, 3: glucosamine hydrochloride, 4: glycine, 5: glutamic acid, 6: Na-glycerophosphate (α- + β-), 7: aspartic acid, 8: serine, 9: alanine, 10: Na-inositolphosphate, 11: chondrosamine hydrochloride, 12: galactose, 13: mannose, 14: rhamnose.

for the serial chromatography detailed by Masamune *et al.*[4] a) The first stage chromatograms are reproduced diagrammatically in Figs. 2A and 2B. In them are given the spots corresponding to ethanolamine, hexosamine, serine and/or glycine, aspartic and/or glutamic acid and glycero- and/or inositol-phosphoric acid. b) A second stage chromatogram showed the presence of ethanolamine, hexosamine, aspartic and glutamic acid and serine (Fig. 2C). c) Fourth stage chromatograms by Solvs. V and VI proved the lead spots (the spots corresponding to 6) in Figs. 2A and 2B were due to glycerophosphate (Figs. 2D–2E). 2. 20 mg. of the substance were hydrolyzed with 2 cc. of 1 *N* H_2SO_4 by heating in an ampoule at 100°C for 4 hours. When cold, the hydrolysate was diluted with 10 cc. of water, freed from sulfuric acid by addition of baryta to pH 6.5–7.0, and thereafter distilled to dryness in vacuo. The residue was dissolved in 0.2 cc. of water and 0.04 cc. portions were sampled for Third stage chromatography. Descending irrigation was effected with *n*-butylacetate-acetic acid-ethanol-water (3:2:1:1) of Masamune and Yosizawa[5] and *n*-butanol-pyridine-water (5:3:2) of Yosizawa.[6] The both chromatograms exhibited the spots corresponding to the positions of galactose, glucosamine and chondrosamine. Fig. 2F is a reproduction of the chromatogram by the latter solvent.

Quantitative analysis. N (micro Kjeldahl) 2.5%. P (a modified Plimmer method[7]) 2.8%. Ash (Pregl method without use of H_2SO_4) 0.3%. Total reduction (Masamune and Tanabe[8]):—28.49 mg. substance was hydrolyzed in an ampoule, by heating with 5 cc. of 1 *N* H_2SO_4 for 4 hours in a boiling water-bath. When cold, the hydrolysate was filtered and 3 cc. of the filtrate were neutralized with 3.15 cc. of 1 *N* KOH. 2 cc. and 4 cc. portions of the neutralized solution were used for the measurement of the total reducing force, which was found to amount to 18.8% of glucose. Amino acids, ethanolamine and hexosamines (The writer is again indebted to Mr. S. Hakomori):—Masamune *et al.*[4] were followed. The substance taken was 7.53 mg. It was hydrolyzed with 0.8 cc. of 6 *N* hydrochloric acid. The hydrolysate was liberated from HCl as in *Paper chromatography* 1. above, and to the dried up residue were added 0.45 cc. of water and 0.05 cc. of 0.2 *N* NaOH. pH of the solution lay between 7.5 and 8.0. Of the centrifuged supernatant 0.05 cc. portions were sampled on paper strips for the chromatographic analysis. The results are shown in Table II. Galactose (Masamune & Ogawa[9]) 13.3%. Here 0.5 cc. of a 0.1% solution in 95% H_2SO_4 was cooled in ice and 0.5 cc. of water and 2.5 cc. of 95% H_2SO_4 containing 1 mg. of thionalide were added to follow the determination procedure. Glycerophosphoric acid:—Since no inorganic nor inositol-phosphoric acid was detected in the acid hydrolysate of the substance (Cf. *Paper chromatography*

TABLE II

Contents of the Water-soluble Components of the Partial Group Lipoid

Component	Content		Molecular ratio
	Amount in 1.5 mg. substance (γ)	% with respect to the Partial Group Lipoid	
Aspartic acid*	29.4	1.96	0.9
Glutamic acid*	76.1	5.08	2.0
Serine*	56.2	3.75	2.1
Hexosamine*	90.8	6.05	2.0
Ethanolamine*	15.7	1.04	1.0
Galactose†		13.3	4.3
Glycerophosphoric acid‡		15.5	5.3

* Masamune *et al.* method. † Masamune & Ogawa method. ‡ Calc. from P content of the substance (Partial Group Lipoid).

1., c)), all the phosphorus must have been bound to form glycerophosphoric acid. Therefore glycerophosphoric acid was calculated from the phosphorus content of the Partial Group Lipoid.

Fr. III. A material (1.1 g.) obtained from 18 g. of the group lipoid was dissolved in alkaline water and after shaking with ether (Only a small part passed into ether), acidified to a pH of about 1.2 and again exhausted with ether. The second ethereal solution (clear brown) was distilled in vacuo to dryness. The residue, dried in a desiccator, was distilled under 1 mg. Hg pressure at 160°C. The distillate which contained a dark splash was taken up in warm abs. alcohol. After centrifuging off of the insoluble dark substance, the solution was distilled in vacuo to dryness and crystallized twice from 75% alcohol. 37 mg. of non-colored crystals were given. The product sintered at 63°C and melted at 64°C and no depression of melting point happened when it was mixed with palmitic acid. N (Lassaigne) and P were not detected. Molecular weight (Rast):

$$M=\frac{8.295\times 40\times 1000}{18.695\times 70}=253 \text{ (Calc. for } CH_3(CH_2)_{14}COOH \quad 256)$$

The most part of the material undistilled under high vacuum (probable unsaturated acids) was found to have resinified, changing insoluble. It was free from N and contained only a trace of P.

Fr. IV. 50 mg. of it were desalted, after dissolving in water, in a Consden, Gordon & Martin apparatus (initial load 75 V and 10 mA, duration 8 hrs.) and after eliminating SO_4-ions, which had diffused in,

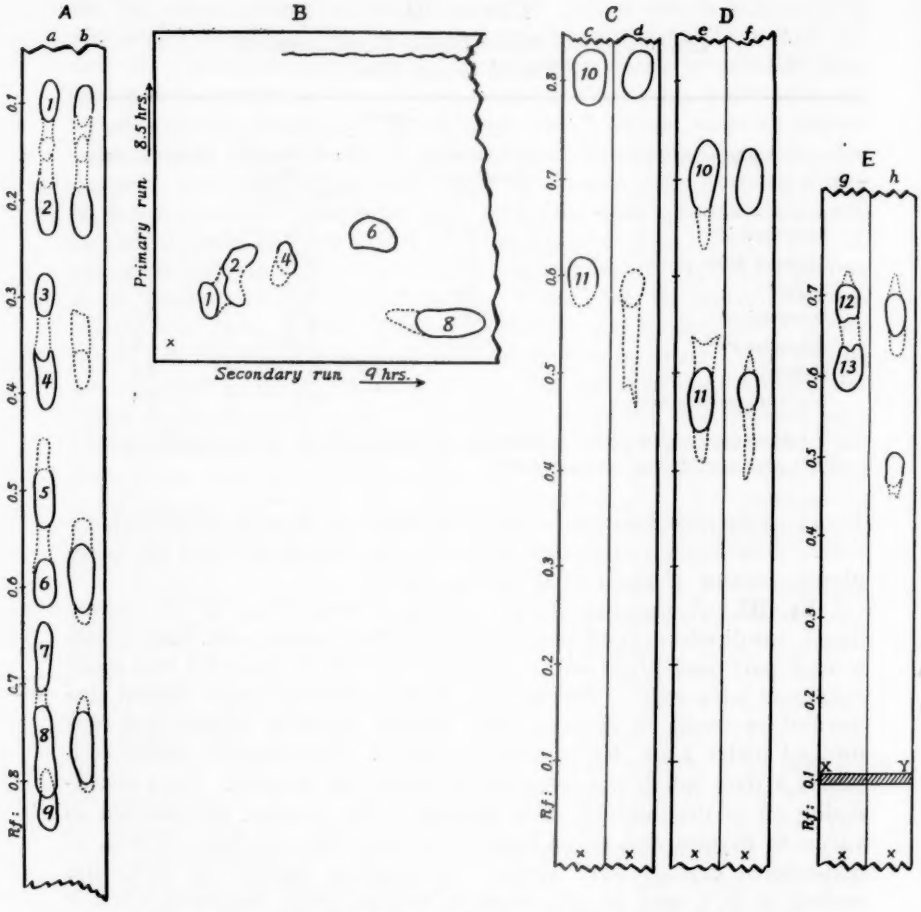

Fig. 3 Chromatograms of Fr. IV. (A) and (B): Second stage chromatograms by (A) descending 80% phenol-ammonia (Solvent front run 50 cm.) and (B) ascending *n*-butanol-acetic acid-water (4:1:2)/80% phenol-NH_3 irrigation. Indicator ninhydrin. Temperature of test 22°±1°C. (C) and (D): Fourth stage chromatograms irrigated with Solvs. V and VI respectively. Solvent fronts run 50 cm. Indicator lead acetate-H_2S. (E): Fifth stage chromatogram. Solv. VI employed. Solvent front run 30 cm. Indicator the same as in (C) and (D). Temperature of tests in (C)–(E) 24° ±1°C.

a, c, e, g: reference runs (c and e: processed with a hydrolysate of a crude inositolphospholipid fraction of brain cephalin[10]). b, B, d, f, h: main runs. 1: aspartic acid, 2: glutamic acid, 3: serine, 4: glycine,

by the aid of baryta, distilled in vacuo to dryness. The still-residue was dissolved in 2 cc. of water and 0.02 cc. portions were sampled for the serial chromatography of Masamune and Hakomori as above. a) The chromatograms were developed with Solvs. I and II and indicated successively with iodine, ninhydrin and lead acetate-hydrogen sulfide or with aniline hydrogen phthalate and Dragendorf reagent. The spots were seen located at positions corresponding to amino acids and glycero- and/or inositol-phosphoric acid, but neither choline nor ethanolamine nor sugars were detected. b) Second stage chromatograms. A one-dimensional descending chromatogram by 80% phenol-NH_3 (Solvent front run 50 cm.) showed spots corresponding to the positions of aspartic and glutamic acid, glycine (a weak spot), alanine (a strong spot) and arginine (a strong spot) (Fig. 3A). Those amino acids were also demonstrated by two-dimensional *n*-butanol-acetic acid-water (4:1:2)/80% phenol-NH_3 chromatography (Fig. 3B). c) The Fourth stage chromatography proved the presence of glycero- and inositol-phosphoric acid (Figs. 3C and 3D). d) Fifth stage chromatography disclosed that the glycerophosphoric acid in c) was the α-isomer (Fig. 3E).

Summary

1. The Group B lipoid of hog lung was subjected to alkaline cleavage and then fractioned.

2. The precipitate occurring on hydrolysis proved to mainly consist of tetracosanol-1.

3. The supernatant fluid was acidified and exhausted with ether. Into ether passed an acetone-insoluble group-active fragment and acetone-soluble organic acids palmitic acid and probable unsaturated acids.

4. The scission products that remained thereby in water were found to be aspartic and glutamic acid, glycine, alanine, arginine and inositol- and α-glycero-phosphoric acid.

5. Its large part precipitated when the group-active, essentially electrophoretically-homogeneous fragment (Partial Group Lipoid) was hydrolyzed with acid, and the supernatant here proved to contain aspartic and glutamic acid, serine, hexosamine (glucosamine and chondrosamine), ethanolamine, galactose and glycerophosphoric acid in molecular proportions of 1:2:2:2:1:4:5.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

5: glucosamine hydrochloride, 6: alanine, 7: histidine, 8: arginine, 9: ethanolamine, 10: barium glycerophosphate, 11: barium inositol-phosphate, 12: Ba-α-glycerophosphate-$Ba(NO_3)_2$ complex, 13: Ba-β-glycerophosphate-$Ba(NO_3)_2$ complex, X—Y: Ba-nitrate band.

References and Notes

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Experimentelle Studien über die Funktion des reticuloendothelialen Systems

I. Mitteilung

Einfluss der Jahreszeiten auf den Kongorotindex

Von

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Seit Aschoff und Landau[1] im Jahre 1913 den Vorschlag machten, eine bestimmte Zellart zu einem System, dem reticuloendothelialen Zellapparat (RES), zusammenzufassen, ist die Herkunft und Funktion des RES immer wieder lebhaft diskutiert worden. Obwohl Adler und Reimann[2] im Jahre 1925 als Funktionsprüfung des RES den Kongorotindex veröffentlichten, doch stimmen noch nicht alle Leute in Bezug auf dessen Verwertbarkeit überein.

Um zu erfahren, ob der Kongorotindex einen Funktionszustand des RES genau zeigen könne, wurde in der vorliegenden Untersuchung zunächst erforscht, wie die Körperbedingungen oder die Jahreszeiten auf den Kongorotindex und die Prothrombinzahl, die als Anzeiger der Lebertätigkeit angenommen wird, einwirken.

Methodik

Unsere Versuchstiere waren ausnahmslos Kaninchen, die 24 Stunden lang vor dem Versuche gehungert hatten. Zur Bestimmung des Kongorotindex nach Adler und Reimann[2] injizierten wir 1 ccm 1%iger Kongorotlösung pro kg Körpergewicht in die Ohrvene und bestimmten kolorimetrisch die Kongorotkonzentration im Serum nach 4 Minuten und nach einer Stunde mit Hilfe des Dubosqschen Apparates. Die Prothrombinzahl im Blut von dergleichen Kaninchen wurde nach Kato'scher Mikromethode[1] bestimmt.

Versuchsergebnisse

1. *Kongorotindex bei normalen Kaninchen*

Bei 431 gesunden Kaninchen erhielten wir als Normalwerte des Kongorotindex zwischen 20 und 80, meistens zwischen 40 und 60, wie aus Tabelle I zu ersehen ist.

Aber der geschlechtliche Unterschied des Kongorotindex ist gar nicht statistisch bedeutsam, weil dessen χ^2 6.49 ist, demnach F^5 (0.05) 2.21 ist (Tabelle I).

TABELLE I

Beziehung des Kongorotindex zum Geschlecht

Geschlecht \ K-index		20—29	30—39	40—49	50—59	60—69	70—79	Total
♂		10	45	97	72	15	3	242
♀		3	29	70	63	19	5	189
Total	Fälle	13	74	167	135	34	8	431
	%	3.1	17.2	38.8	31.4	7.9	1.9	100.0

TABELLE II

Beziehung des Kongorotindex zum Körpergewicht

Körpergewicht \ K-index	20—29	30—39	40—49	50—59	60—69	70—79	Total
1.30–1.49		2	3	3	1		9
1.50–1.69	1	8	15	6	2		32
1.70–1.89	3	26	35	27	2	1	94
1.90–2.09	4	19	57	30	10	1	121
2.10–2.29	3	11	31	32	12	4	93
2.30–2.49	1	6	18	27	5	1	58
2.50–2.69	1	1	7	8	1	1	19
2.70–2.89		1	1	2	1		5
Total	13	74	167	135	34	8	431

TABELLE III

Jahreszeitliche Schwankungen des Kongorotindex

Jahreszeiten \ K-index	20—29	30—39	40—49	50—59	60—69	70—79	Total
Frühling	4	17	50	30	10	4	115
Sommer	2	16	43	62	19	4	146
Herbst	1	9	25	24	4		63
Winter	6	32	49	19	1		107
Total	13	74	167	135	34	8	431

Betrachten wir in Tabelle II die Beziehung des Kongorotindex zum Körpergewicht des Kaninchens, so gibt es kaum bedeutsamen Unterschied, da der Korrelationskoeffizient +0.1781 ist und $t\sigma$ 3.7488 ist.

2. *Beziehung der Jahreszeiten zum Kongorotindex*

Die Temperatur des Frühlings in der Sendai-Gegend ist 10–19°C, die des Sommers über 20°C, die des Herbstes 10–19°C und die des Winters unter 9°C.

Wie aus Tabelle III zu sehen, ist der Einfluss der Jahreszeiten auf den Kongorotindex mathematisch-statistisch vom bedeutsamen Unterschied, weil F_0 grösser ist als F^5_∞ (0.01), indem x^2 54.95 ist, F_0 3.77 ist und F_∞ (0.01) 2.03 ist. Betrachten wir weiter diese bedeutsamen Differenzen in Einzelheiten, sind x^2 des Winters (28.76) und des Sommers (19.21) bedeutsam, aber die des Frühlings (3.90) und des Herbstes (3.08) sind nicht bedeutsam, weil es F^{15}_∞ (0.05) 1.67 darstellt, indem es statistisch verzeihliches x^2 25.06 sein soll. Vor allem ist es klar geworden, dass der Kongorotindex von 30–39 zu häufig ist und von 50–69 zu gering im Sommer, dagegen ist der Wert von 30–39 zu häufig und der von 50–60

TABELLE IV

Kongorotindex bei Kongorotbelastungsversuchen in der kurzen Zeitspanne

Jahreszeiten	Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)		
			1	3	5
Frühling	1	60.4	61.4	66.7	68.2
	2	52.0	54.8	56.3	56.7
	3	44.4	53.7	48.3	44.4
	4	41.5	40.8	42.8	36.0
	Durchschnitt	50.0	53.2	54.3	53.7
	Differenz		3.2	4.3	3.7
Sommer	1	59.7	72.2	82.8	72.2
	2	47.7	60.0	66.7	69.0
	3	44.4	54.8	65.8	61.5
	4	43.1	55.6	58.1	59.3
	Durchschnitt	48.3	60.1	67.4	65.8
	Differenz		11.8	19.1	17.5
Herbst	1	52.3	53.0	65.0	65.6
	2	46.3	53.3	52.5	57.0
	3	35.5	38.5	50.0	55.5
	4	30.1	35.5	40.4	52.7
	Durchschnitt	40.8	45.3	51.8	57.3
	Differenz		4.5	11.0	16.5
Winter	1	50.0	69.8	56.7	55.6
	2	46.8	54.8	56.3	46.7
	3	44.5	58.8	60.5	57.7
	4	41.5	58.0	53.5	58.9
	Durchschnitt	45.8	59.6	58.5	56.0
	Differenz		13.8	12.7	10.2

TABELLE V

Kongorotindex bei Kongorotbelastungsversuchen im längeren Intervall

Jahreszeiten	Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)					
			3	5	8	12	24	48
Frühling	1	55.2	63.5	68.3	54.7	58.0	50.0	55.0
	2	45.7	48.6	50.6	46.4	49.5	44.7	43.0
	3	40.4	41.5	40.1	50.9	40.4	44.8	42.0
	4	40.2	50.0	48.2	48.4	39.0	46.4	44.3
	5	32.6	42.8	44.1	38.9	33.7	38.7	40.0
	Durchschnitt	43.8	50.8	52.3	50.3	46.1	46.5	46.2
	Differenz		7.0	8.5	6.5	2.3	2.7	2.4
Sommer	1	62.6	66.3	66.7	69.4	64.5	58.8	65.8
	2	54.5	57.2	60.0	60.0	55.7	58.2	54.0
	3	37.5	49.9	56.6	50.0	40.7	44.3	54.8
	4	35.1	44.6	58.2	47.7	56.3	53.3	46.1
	Durchschnitt	47.3	54.1	60.8	58.1	54.8	54.1	56.1
	Differenz		6.8	13.5	10.8	7.5	6.8	8.8
Herbst	1	52.8	51.2	55.7	46.6	50.0	54.5	50.0
	2	41.5	43.4	47.5	42.4	47.5	39.0	43.3
	3	39.4	40.3	43.9	45.6	32.7	41.9	38.7
	Durchschnitt	44.8	45.0	49.2	44.8	43.6	44.9	44.1
	Differenz		0.2	4.4	0	−1.2	0.1	−0.7
Winter	1	46.0	55.7	48.7	52.8	50.4	53.5	45.0
	2	45.1	62.4	51.3	50.8	53.3	41.0	43.6
	3	37.2	55.7	44.8	39.2	37.7	37.3	37.0
	4	36.5	36.7	43.3	43.5	44.1	44.5	33.3
	Durchschnitt	41.9	51.3	46.7	46.3	47.6	44.1	39.8
	Differenz		9.4	4.8	4.4	5.7	2.2	2.1

zu gering im Winter.

Daraus folgt, dass der Kongorotindex im Sommer aufzusteigen und im Gegensatz dazu im Winter herabzufallen neigt.

3. *Einfluss der Jahreszeiten auf Kongorotbelastungsversuche*

Wenn man den Kongorotindex eines Kaninchens aufeinanderfolgend untersuchen will, so soll man eine Veränderung der Aufnahmefähigkeit für injizierten Kongorot der reticuloendothelialen Zellgruppen in Betracht ziehen. Deshalb ist zunächst genau zu erforschen, wie 4 Jahreszeiten deren Einfluss auf die Kongorotbelastungsversuche geben.

Es ergibt sich aus unseren Versuchen, wie aus Tabelle IV und V zu ersehen, dass der Kongorotindex sich im Sommer und Winter deutlich vermehrt, sowohl in der kürzeren Zeitspanne nach aufeinanderfolgenden Belastungen, als auch in dem längeren Intervall.

Betreffs der Schwankung der Prothrombinzahl, die in dergleichen Weise wie beim Kongorotindex bestimmt wurde, zeigt sich die Neigung, dass sie sich im Sommer vermindert und im Herbst vermehrt, obwohl

TABELLE VI

Prothrombinzahl bei Kongorotbelastungsversuchen in der kurzen Zeitspanne

Jahreszeiten	Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)		
			1	3	5
Frühling	1	1.00	1.01	1.04	1.05
	2	1.00	1.01	1.03	1.03
	3	1.00	1.01	1.02	1.02
	4	1.00	1.00	1.02	1.02
	Durchschnitt	1.00	1.01	1.03	1.03
	Differenz		0.01	0.03	0.03
Sommer	1	1.00	1.00	1.01	1.05
	2	1.00	0.97	0.95	0.95
	3	1.00	1.00	0.95	0.94
	Durchschnitt	1.00	0.99	0.97	0.98
	Differenz		–0.01	–0.03	–0.02
Herbst	1	1.00	1.04	1.07	1.07
	2	1.00	0.99	1.01	1.05
	3	1.00	1.01	1.01	1.02
	4	1.00	0.99	0.97	1.01
	Durchschnitt	1.00	1.01	1.02	1.04
	Differenz		0.01	0.02	0.04
Winter	1	1.00	1.00	1.02	1.02
	2	1.00	1.01	0.98	1.03
	3	1.00	0.97	0.97	1.02
	4	1.00	1.02	1.00	1.00
	Durchschnitt	1.00	1.00	0.99	1.02
	Differenz		0	–0.01	0.02

ihre Schwankung viel geringer ist (Tabelle VI und VII).

DISKUSSION

Als Prüfungsmethoden der Funktion des RES sind bis jetzt mehrere Verfahren von verschiedenen Autoren vorgeschlagen worden (Eppinger und Stöhr[4], Paschkis[5], Saxl und Donath[6], Adler und Reimann[2], Leites und Riabow[7]). Aber es ist allgemein anerkannt, dass der Kongorotindex nach Adler und Reimann[2] am bequemsten und zuverlässigsten ist.

In Bezug auf das Geschlecht des Kaninchens berichtet Kinoshita[8] über keine Differenz des Kongorotindex, aber Kinoshita[8] und Miyoshi[9] betonen, dass der Kongorotindex der jüngeren Kaninchen höher ist als derjenige der ausgewachsenen.

Wie es aus unseren Versuchsergebnissen ersichtlich ist, hat der Kongorotindex in Bezug auf Geschlecht und Körpergewicht des Kaninchens kaum bedeutsame Differenz.

Es ist schon allgemein bekannt, dass die Glykogenbildungsfähigkeit

TABELLE VII

Prothrombinzahl bei Kongorotbelastungsversuchen im längeren Intervall

Jahreszeiten	Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)					
			3	5	8	12	24	48
Frühling	1	1.00	1.00	0.99	1.05	1.02	1.01	1.00
	2	1.00	1.03	1.03	1.01	0.98	1.01	0.98
	3	1.00	1.01	0.97	0.98	1.03	0.97	0.94
	4	1.00	1.01	0.99	1.01	0.97	0.99	0.96
	5	1.00	0.95	0.98	1.01	1.01	1.01	0.95
	Durchschnitt	1.00	1.00	0.99	1.01	1.00	1.00	0.97
	Differenz		0	–0.01	0.01	0	0	–0.03
Sommer	1	1.00	1.04	1.01	1.02	0.98	1.00	0.98
	2	1.00	0.96	0.96	1.00	1.03	1.00	1.01
	Durchschnitt	1.00	1.00	0.99	1.01	1.00	1.00	1.00
	Differenz		0	–0.01	0.01	0	0	0
Herbst	1	1.00	1.00	0.96	1.04	1.08	1.02	0.99
	2	1.00	1.05	1.04	1.07	1.06	1.04	1.01
	3	1.00	1.02	1.03	1.03	1.01	0.95	0.93
	Durchschnitt	1.00	1.02	1.01	1.05	1.05	1.00	0.97
	Differenz		0.02	0.01	0.05	0.05	0	–0.03
Winter	1	1.00	1.00	0.99	1.01	1.07	1.00	0.95
	2	1.00	1.01	1.01	1.06	1.02	1.05	0.98
	3	1.00	0.96	1.00	1.00	1.04	1.04	1.04
	4	1.00	0.99	1.00	1.04	1.03	1.04	0.99
	Durchschnitt	1.00	0.99	1.00	1.03	1.04	1.03	0.99
	Differenz		–0.01	0	0.03	0.04	0.03	–0.01

der Leber im Sommer und Winter beeinträchtigt, dagegen im Frühling und Herbst gesteigert wird (Kimura[10], Musha[11]). Ueberdies bemerkte So[12] eine Herabsetzung des Ausscheidungsvermögens des Azorubin-S von Leber im Sommer.

Nach unseren Resultaten vermehrt sich der Kongorotindex im Sommer und vermindert sich im Winter, und die Blockade nach Kongorotbelastungsproben erfolgt viel deutlicher im Sommer und Winter, doch im Frühling und Herbst geringen Grades. In dergleichem Sinne wie beim Kongorotindex verändert sich die Prothrombinzahl in 4 Jahreszeiten, obwohl ihre Schwankungen viel geringer sind.

Daraus wird es natürlich zu dem Schluss gezwungen, dass man beim Kaninchenversuche einen jahreszeitlichen Einfluss auf Kongorotindex und Prothrombinzahl im Betracht ziehen soll.

ZUSAMMENFASSUNG

Aus den Ergebnissen der obenerwähnten Untersuchungen von 431 Kaninchen ist das Folgende abzuleiten:

1) Der Kongorotindex bei normalen Kaninchen liegt meistens zwischen 40 und 60.

2) Der Kongorotindex zeigt kaum bedeutende Beziehung auf Geschlecht und Körpergewicht des Kaninchens.

3) Der Kongorotindex neigt im Sommer aufzusteigen, doch im Winter herabzufallen.

4) Bei Kongorotbelastungsproben vermehrt sich der Kongorotindex viel deutlicher im Sommer und Winter, dagegen im Frühling und Herbst viel geringer. In dergleichen Proben neigt die Prothrombinzahl im Sommer sich zu vermindern und im Herbst zu vermehren, obwohl ihre Schwankungen viel geringer sind.

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Experimentelle Studien über die Funktion des reticuloendothelialen Systems

II. Mitteilung

Beziehung der Funktion der Kupfferschen Sternzellen zur Leberfunktion, mit besonderer Berücksichtigung des Einflusses der Zuckerbehandlung

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Wenn man nach Rössle[1] als Hepaton die zu einer Gallenkapillare gehörigen Leberzellbalken samt zugehörigem Reticuloendothel zu bezeichenen annimmt, kann man unter Leberfunktion die Summe der Funktion des epithelialen und des vaskulären Parenchyms verstehen. Bekanntlich aber ist die phagozytäre Fähigkeit der Kupfferschen Sternzellen am stärksten von den reticuloendothelialen Zellgruppen (Kiyono[2], Kinoshita[3]).

Infolgedessen richtet sich unsere Untersuchung darauf, ob man die Funktion der Kupfferschen Sternzellen von der Leberfunktion getrennt betrachten kann, und ob der Kongorotindex den Funktionszustand des RES einschliesslich der Kupfferschen Sternzellen genau veranschaulich machen kann. Auch in der vorliegenden Untersuchung bestimmten wir die Prothrombinzahl als Funktionsanzeiger der Lebertätigkeit anstatt der übrigen Chromodiagnostik der Leberfunktion.

Die Auswahl der Versuchstiere sowie Methodik war die gleiche wie bei der I. Mitteilung[4] und wir verglichen den Durchschnittswert von 2 oder 3 Kaninchen mit den Belastungsversuchen in der I. Mitteilung[4].

Versuchsergebnisse

1. *Blockierungsversuche des RES*

Als Blockierungsmittel des RES benutzten wir Tusche oder kolloidales Silber. Wenn man 10 ccm 3% iger Tusche pro kg in die Ohrvene einführt, so vermehrt sich, wie Tabelle I zeigt, der Kongorotindex um 40.8

TABELLE I

Blockierungsversuche des RES

		Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)					
				3	5	8	12	24	48
Tusche	K-index	1	46.8	91.4	75.6	63.2	65.5	65.0	64.3
		2	40.0	80.0	50.0	56.0	41.0	43.5	36.5
		Durchschnitt	42.8	83.8	58.7	59.3	51.8	53.4	46.3
		Differenz		40.8	11.5	16.5	10.2	10.5	4.2
	P-zahl	1	1.00	0.98	0.97	0.99	0.98	1.03	1.04
		2	1.00	1.00	1.04	0.96	1.05	1.03	1.02
		Durchschnitt	1.00	0.99	1.00	0.97	1.02	1.03	1.03
		Differenz		–0.03	–0.01	–0.08	–0.03	0.03	0.06
Kolloidales Silber	K-index	1	49.1	85.2	67.5	74.0	63.7	59.2	50.0
		2	31.1	74.0	93.3	72.8	66.6	53.4	63.2
		Durchschnitt	38.0	78.5	83.6	63.2	65.3	56.2	56.8
		Differenz		40.3	41.2	25.2	28.5	18.1	19.5
	P-zahl	1	1.0	1.00	1.00	1.05	1.06	1.03	1.04
		2	1.0	1.04	0.99	0.98	0.97	0.97	1.01
		Durchschnitt	1.0	1.02	1.00	1.02	1.00	1.00	1.03
		Differenz		0	–0.01	–0.03	–0.05	0	0.06

nach 3 Stunden, trotzdem die Prothrombinzahl sich nach 8 Stunden ganz gering vermindert. Diese Verhältnisse sind bei intravenöser Injektion von 10 ccm 1% igen kolloidalen Silbers pro kg viel deutlicher zu sehen als im Falle der Tuscheblockade, indem eine Steigerung des Kongorotindex 48 Stunden lang andauert, während die Prothrombinzahl fast unverändert bleibt.

Deshalb vermehrt sich der Kongorotindex vorübergehend bei der Blockade des RES, aber die Prothrombinzahl bleibt dabei fast unverändert.

2. *Stimulierungsversuche des RES*

Wie man aus Tabelle II ersieht, vermindert sich der Kongorotindex nach der Stimulierung des RES durch subkutane Communininjektion (Shwartzman'sches Filtrat) in Menge von 1 ccm pro kg, während die Prothrombinzahl fast unverändert bleibt. Injiziert man 1 ccm 50% iger Natriumthiosulfatlösung pro kg unter die Haut, so vermindert sich der Kongorotindex allein um 13.6.

Daraus folgt, dass der Kongorotindex sich bei der Stimulierung des RES vermindert, aber die Prothrombinzahl dabei nicht beeinflusst wird.

3. *Schädigungsversuche der Leber*

Bei subkutaner Chloroforminjektion in Menge von 0.25 ccm pro kg vermehrt sich, wie Tabelle III zeigt, der Kongorotindex allmählich und die Prothrombinzahl vermindert sich auch deutlich. Wenn man 0.5

TABELLE II

Stimulierungsversuche des RES

		Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)		
				1	3	5
Communin	K-index	1	72.1	66.7	75.0	73.5
		2	52.7	55.0	56.5	59.8
		Durchschnitt	60.6	59.8	65.0	65.8
		Differenz		−12.6	−14.7	−12.3
	P-zahl	1	1.00	1.01	0.95	0.94
		2	1.00	0.96	0.96	0.98
		Durchschnitt	1.00	0.99	0.96	0.96
		Differenz		0	−0.01	−0.02
Natrium-thiosulfat	K-index	1	53.2	56.5	66.7	50.0
		2	39.6	39.3	29.9	19.1
		Durchschnitt	48.2	51.1	55.3	45.6
		Differenz		−10.9	− 5.6	−13.6
	P-zahl	1	1.00	1.03	1.00	1.02
		2	1.00	0.98	0.98	1.05
		Durchschnitt	1.00	1.01	0.99	1.04
		Differenz		0.01	0	0.02

% ige Phosphor-Olivenöllösung in Menge von 0.75 ccm pro kg subkutan injiziert, so findet man eine Erhöhung des Kongorotindex nach anfänglicher Herabsetzung, während eine Verminderung der Prothrombinzahl etwas geringgradiger ist als bei Chloroform. Die totale Choledochusligatur gleich nach der Laparotomie verursachte eine auffallende Vermehrung des Kongorotindex und sogar eine bemerkenswerte Verminderung der Prothrombinzahl. Aber die obengenannten Veränderungen geschehen bei den 2 Leberlappenresektionen (24.8∼41.2%) ganz leichtgradig.

Aus obenerwähnten Versuchen ist es ersichtlich, dass der Kongorotindex sich bei verschiedenarten Leberschädigungen vermehrt, aber die Prothrombinzahl im Gegensatz dazu sich vermindert.

4. *Verstärkungsversuche der Leberfunktion*

Als stimulierende Mittel der Lebertätigkeit werden Glukose, Lävulose, Yakriton, Thyroxin, Lecithin usw. von verschiedenen Autoren angegeben. Aus Tabelle IV sieht man, dass die intravenöse Injektion von 10 ccm 25 % iger Glukose oder Lävulose pro kg eine Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl hervorruft. Wenn man Yakriton (das Entgiftungshormon der Leber) in Menge von 0.5 Einheit pro kg unter die Haut injiziert, so vermindert sich der Kongorotindex zwar anfangs, aber er vermehrt sich später, während die Prothrombinzahl sich sofort nach der Injektion erhöht, dann allmählich sich herabsetzt. Dagegen werden der Kongorotindex und die Prothrombinzahl bei subkutaner Thyroxininjektion in Menge von 0.5 ccm oder intravenöser Injektion

TABELLE III

Schädigungsversuche der Leber

		Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)					
				3	5	8	12	24	48
Chloroform	K-index	1	46.9	58.8	58.8	69.5	85.6	+	
		2	39.0	53.3	58.9	75.0	70.2	85.6	+
		Durchschnitt	43.0	55.7	58.3	72.3	77.0		
		Differenz		3.3	10.5	24.9	28.3		
	P-zahl	1	1.00	0.98	0.96	0.88	0.72	0.51+	
		2	1.00	1.01	0.98	0.82	0.76	0.73	+
		Durchschnitt	1.00	0.99	0.97	0.85	0.74	0.62	
		Differenz		0	−0.03	−0.18	−0.30	−0.41	
Phosphor	K-index	1	40.0	39.2	44.0	50.0	57.6	51.2	57.6
		2	38.1	43.1	54.3	53.3	60.6	53.6	56.8
		Durchschnitt	39.2	41.0	50.3	51.6	59.0	52.5	57.3
		Differenz		− 7.6	6.3	8.0	14.1	11.1	20.2
	P-zahl	1	1.00	0.98	0.99	0.97	0.91	0.88	0.72
		2	1.00	0.97	1.00	0.97	0.93	0.84	0.80
		Durchschnitt	1.00	0.98	1.00	0.97	0.92	0.86	0.76
		Differenz		−0.01	0	−0.06	−0.12	−0.17	−0.25
Laparotomie	K-index	1	71.3	72.6	72.8	78.3	70.3	62.2	60.0
		2	50.0	53.5	65.0	53.7	64.4	52.7	55.2
		3	47.0	50.0	58.2	62.6	59.2	54.2	44.2
		Durchschnitt	55.8	57.6	65.8	62.7	65.0	51.7	52.4
		Differenz		1.8	10.0	6.9	9.2	− 4.1	− 3.4
	P-zahl	1	1.00	1.01	0.99	1.00	1.01	1.01	1.03
		2	1.00	1.02	1.00	1.02	1.02	1.01	1.00
		3	1.00	1.00	1.00	1.00	0.99	0.97	0.95
		Durchschnitt	1.00	1.01	1.00	1.01	1.01	1.00	0.99
		Differenz		0.01	0	0.01	0.01	0	−0.01
Choledochusligatur	K-index	1	57.2	80.1	80.0	87.5	83.3	+	
		2	55.2	72.3	91.7	95.8	62.6	+	
		3	53.0	79.8	88.2	83.5	75.2	88.2	94.5
		Durchschnitt	55.3	79.0	85.7	88.9	77.0		
		Differenz		21.9	20.4	26.7	12.5		
	P-zahl	1	1.00	0.93	0.90	0.89	0.87	+	
		2	1.00	0.61	0.63	0.64	0.64	+	
		3	1.00	0.90	0.89	0.86	0.85	0.85	0.82
		Durchschnitt	1.00	0.82	0.81	0.79	0.78		
		Differenz		−0.19	−0.19	−0.22	−0.23		
Leberlappenresektion	K-index	1	57.8	61.6	69.9	75.0	71.0	60.6	66.5
		2	48.7	77.1	77.7	80.2	66.7	60.6	57.3
		Durchschnitt	52.8	68.5	73.3	77.8	68.3	60.6	61.7
		Differenz		13.9	11.0	18.1	6.3	11.8	12.3
	P-zahl	1	1.00	0.81	0.93	0.90	0.90	0.90	0.93
		2	1.00	0.97	0.97	0.99	0.98	0.92	1.03
		Durchschnitt	1.00	0.89	0.95	0.95	0.94	0.91	0.98
		Differenz		−0.12	−0.05	−0.06	−0.07	−0.09	−0.01

TABELLE IV

Verstärkungsversuche der Leberfunktion

		Nr. der Kaninchens	Vor Injektion	Zeit nach Injektion (St.)					
				3	5	8	12	24	48
Glukose	K-index	1	60.1	70.1	70.8	59.0	60.0	46.8	52.6
		2	53.3	55.0	55.0	57.6	57.8	46.5	52.2
		Durchschnitt	57.2	62.7	65.0	58.4	58.8	46.7	52.3
		Differenz		− 1.3	− 5.7	− 9.6	− 5.9	−17.3	−13.7
	P-zahl	1	1.00	0.99	1.06	1.10	1.10	1.05	1.06
		2	1.00	0.99	0.96	1.00	1.02	1.12	1.07
		Durchschnitt	1.00	0.99	1.01	1.05	1.06	1.08	1.06
		Differenz		−0.01	0.02	0.04	0.06	0.08	0.06
Lävulose	K-index	1	50.0	46.4	54.0	66.7	37.5	37.4	50.0
		2	48.0	50.8	59.8	40.0	59.2	57.4	53.2
		Durchschnitt	48.9	48.7	57.1	49.8	47.1	45.7	51.4
		Differenz		− 7.0	− 5.3	− 9.9	− 9.1	−10.0	− 6.3
	P-zahl	1	1.00	1.11	1.04	0.99	1.06	1.00	1.05
		2	1.00	1.05	1.10	1.08	1.09	1.09	1.05
		Durchschnitt	1.00	1.08	1.07	1.03	1.07	1.04	1.05
		Differenz		0.08	0.08	0.02	0.07	0.04	0.05
Yakriton	K-index	1	35.3	33.4	41.4	46.2	38.5	41.3	45.1
		2	33.8	29.5	35.2	35.9	35.0	35.3	52.1
		Durchschnitt	34.6	31.4	38.2	40.9	37.0	38.6	48.5
		Differenz		−12.6	− 1.2	1.9	− 3.3	1.8	16.0
	P-zahl	1	1.00	1.10	0.99	0.95	0.91	0.89	0.90
		2	1.00	1.03	1.01	0.96	0.94	0.96	0.91
		Durchschnitt	1.00	1.07	1.00	0.96	0.93	0.93	0.91
		Differenz		0.08	0	−0.07	−0.11	−0.11	−0.08
Thyroxin	K-index	1	52.2	52.3	48.8	40.5	40.5	35.9	26.9
		2	37.6	48.8	43.8	45.0	46.0	40.3	20.3
		Durchschnitt	45.0	50.5	46.3	42.8	45.7	38.0	23.9
		Differenz		− 3.9	− 3.5	− 6.6	− 5.0	− 9.7	−23.2
	P-zahl	1	1.00	0.90	0.89	0.89	0.98	1.00	1.01
		2	1.00	0.82	0.88	0.89	1.01	1.02	1.02
		Durchschnitt	1.00	0.86	0.89	0.89	1.00	1.01	1.02
		Differenz		−0.13	−0.11	−0.14	−0.04	−0.02	0.03
Lecithin	K-index	1	36.6	47.9	48.9	45.3	34.2	32.3	38.3
		2	34.0	38.5	44.5	50.0	44.9	43.7	46.3
		Durchschnitt	35.3	43.0	46.4	47.6	39.3	37.7	41.9
		Differenz		0.9	− 2.4	1.5	− 3.5	− 4.4	− 2.2
	P-zahl	1	1.00	0.95	0.93	0.94	1.00	1.03	1.00
		2	1.00	0.91	0.92	1.05	1.00	0.99	0.98
		Durchschnitt	1.00	0.93	0.93	1.01	1.00	1.01	0.99
		Differenz		−0.07	−0.06	0	0	0.01	−0.01

von 2 ccm 3% iger Lecithinlösung pro kg herabgesetzt.

Aus diesen Ergebnissen ist es also klar, dass Glukose und Lävulose eine langdauernde, aber Yakriton nur eine anfängliche Steigerung beider

Frunktion des RES und Leberparenchyms hervorrufen, während Thyroxin und Lecithin nur das RES stimulieren können.

5. *Zuckerversuche mit verschiedenen Vitaminen*

Wenn man 10 ccm 25 % iger Glukose oder Lävulose pro kg kombiniert mit Redoxon (Vitamin C) in Menge von 50 mg pro kg, Kativ (Vitamin K) in Menge von 10 mg pro kg oder Hesperin (Vitamin P) in Menge von 50 mg pro kg in die Ohrvene injiziert, so konstatiert man, wie Tabelle V zeigt, eine deutliche Herabsetzung des Kongorotindex.

Deshalb ist es kalr, dass die obenerwähnten Vitamine die Wirkung

TABELLE V

Kongorotindex bei Zuckerversuchen mit verschiedenen Vitaminen

	Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)		
			1	3	5
Glukose	1	63.5	66.5	69.3	80.0
	2	52.0	57.5	48.4	82.8
	Durchschnitt	57.5	62.6	59.7	81.3
	Differenz		- 6.7	-16.9	6.3
Glukose mit Vitamin C	1	62.5	60.8	64.0	62.5
	2	50.0	51.6	51.0	60·0
	Durchschnitt	56.0	55.5	56.3	61.3
	Differenz		-12.3	-18.8	-12.3
Glukose mit Vitamin K	1	68.1	53.5	60.0	67.3
	2	53.5	50.0	59.4	66.6
	Durchschnitt	60.0	52.1	60.1	66.8
	Differenz		-19.7	-19.0	-10.7
Glukose mit Vitamin P	1	35.7	25.0	23.8	21.1
	2	32.2	32.2	30.4	33.8
	Durchschnitt	34.4	30.0	27.7	27.4
	Differenz		-18.2	-19.4	-17.2
Lävulose	1	48.7	69.3	66.6	59.0
	2	47.7	56.2	55.3	59.3
	Durchschnitt	48.2	63.2	59.8	59.0
	Differenz		4.2	- 7.5	- 6.5
Lävulose mit Vitamin C	1	61.6	64.5	66.5	74.0
	2	43.9	44.0	54.8	55.9
	Durchschnitt	52.8	54.2	62.8	65.0
	Differenz		-10.4	- 9.1	- 5.3
Lävulose mit Vitamin K	1	56.8	60.0	62.3	68.0
	2	51.1	61.1	64.1	64.9
	Durchschnitt	54.2	60.5	63.2	66.4
	Differenz		- 5.5	-10.1	- 7.3
Lävulose mit Vitamin P	1	54.0	50.0	34.7	42.8
	2	38.8	44.5	44.3	44.5
	Durchschnitt	45.0	47.1	39.4	43.6
	Differenz		-11.7	-18.3	-11.6

der Glukose und Lävulose auf die Funktion des RES verstärkend einwirken können.

Diskussion

Es ist allgemein anerkannt, dass das Kongorot, das aus hochkolloidalem Farbstoff mit geringer Diffusibilität besteht, primär vom RES bei intravenöser Injektion aufgenommen, dann ein grosser Teil von ihm langsam in der Galle ausgeschieden, jedoch im Harn normalerweise nur geringe Spuren nachgewiesen wird (Adler und Reimann[6], Wilensky[6], Yano[7], Takeda[8], Kinoshita[8]). Nach mehreren Autoren ist die ausgeschiedene Menge des Kongorots durch Kaninchenleber sehr gering, z. B. Takeda[8] bericht 5.1 % in 4 Stunden, Yano[7] 18 % in denselben Stunden, doch Kinoshita[8] 13.5 % in 1 Stunde und 33 % in 3 Stunden. Dagegen liegt der Kongorotindex meistens zwischen 40 und 60, wie in der I. Mitteilung[4] berichtet. Daraus folgt, dass ein grosser Teil des injizierten Kongorots anfangs vom RES aufgenommen wird. Aber Meinungen der Autoren sind darüber geteilt, welche von beiden, Leberzellen und Sternzellen, an der Ausscheidung des Kongorots in der Galle sich beteiligt: einerseits legen Goldmann[9], Möllendorff[10] und Kinoshita[3] grosses Gewicht auf Sternzellen, doch andererseits verleiht Tada[11] Leberzellen erhöhte Bedeutung. Infolgedessen glauben mehrere Autoren wie Adler und Reimann[5], Wilensky[6], Muda[12], Kinoshita[3], Nagashima[13], Miyoshi[14], Tsurui[15], dass der Kongorotindex die Funktion des RES zu verraten imstande ist, doch behauptet Ninomiya[16], dass es sich nur um die Leberfunktion handelt.

In Bezug auf Prothrombin, das unter Einwirkung des Vitamins K hauptsächlich in der Leber gebildet wird, bericht Imanaga[16], dass es auf das RES sich bezieht, aber Warner[17] und Franklin[18] behaupten eine engere Beziehung mit Leberzellen.

Bekanntlich wird die Blockade des RES im allgemeinen von Tusche oder kolloidalem Silber durchgeführt. In neuerer Zeit bericht Iwatsuki[19] als Stimulant des RES Communin (Shwartzman'sches Filtrat) und Kawaguchi[20] Natriumthiosulfat.

Nach unserer Versuchsergebnissen wird es kalr, dass der Kongorotindex ein Anzeiger der Funktion des RES ist, indem der Kongorotindex sich bei der Blockade des RES erhöht und bei der Reizung des RES sich herabsetzt, während die Prothrombinzahl ganz unverändert verläuft.

Von den verschiedenartigen Lebergiften schädigt Chloroform hauptsächlich vom Zentrum des Leberparenchyms ab, im Gegenteil Phosphor hauptsächlich von der Peripherie desselben ab. Weiterhin berichten Matsuo und seine Schüler[21], dass die Unterbindung des Choledochus

eine schwere Leberschädigung herbeiführt, und Kitani[22] behauptet, dass die partielle Lappenresektion der Leber nur eine vorübergehende Schädigung veranlasst.

Nach bisherigen Arbeiten aus unserem Institut ist es schon begreiflich, dass Glukose sowie Lävulose ein Verstärkungsmittel der Leber ist. Als Stimulant der Lebertätigkeit wird auch Yakriton, das Entgiftungshormon der Leber, von Sato und seinen Schülern[23], Thyroxin und Lecithin von Matsuo und seinen Schülern[21] empfohlen.

Aus unseren diesbezüglichen Ergebnissen ist ersichtlich, dass obige Leberschädigungen eine Steigerung des Kongorotindex und eine Herabsetzung der Prothrombinzahl, aber Reizung der Lebertätigkeit durch Glukose, Lävulose und Yakriton eine Erniedrigung des Kongorotindex und eine Erhöhung der Prothrombinzahl verursachen, während Thyroxin und Lecithin eine Herabsetzung des Kongorotindex und der Prothrombinzahl veranlasst.

Daraus ist zu vermuten, dass der Kongorotindex bei Funktionsstörung des RES, in beiden Fällen, wo es primär oder sekundär nach Leberveränderung geschädigt wird, sich vermehrt, während die Prothrombinzahl vielmehr einen Funktionszustand der Leberzellen darstellt als den des RES. Deshalb sind Thyroxin und Lecithin als Stimulant des RES, nicht als Reizmittel der Leberzellen zu betrachten.

Nach Koster *et al.*[21] wird es histologisch nachgewiesen, dass die intravenös injizierte Glukose die Sternzellen stimuliert. In dergleichen Weise bemerken wir eine mässige Herabsetzung des Kongorotindex, besonders bei Kombination mit Vitamin C, K und P.

Also ist es ersichtlich, dass hier die Reizung des RES als einer der Wirkungsmechanismen der Zuckerbehandlung mitwirken kann.

Zusammenfassung

Fassen wir obige Versuchsergebnisse zusammen, so können wir wie folgend schliessen:

1) Bei der Blockade des RES durch Tusche und kolloidales Silber steigert sich der Kongorotindex und wird es bei der Reizung des RES durch Communin (Shwartzman'sches Filtrat) und Natriumthiosulfat herabgesetzt, während die Prothrombinzahl fast unbeeinflusst bleibt.

2) Bei Leberschädigungen durch Chloroform, Phosphor, Ligatur des Choledochus und Lappenresektion der Leber werden eine Steigerung des Kongorotindex und eine Herabsetzung der Prothrombinzahl herbeigeführt.

3) Bei Stimulierung der Lebertätigkeit durch Glukose, Lävulose und Yakriton (das Entgiftungshormon der Leber) werden eine Verminderung des Kongorotindex und eine Erhöhung der Prothrombinzahl verur-

sacht, aber Thyroxin und Lecithin setzt den Kongorotindex und die Prothrombinzahl herab.

4) Redoxon (Vitamin C), Kativ (Vitamin K) und Hesperin (Vitamin P) sind imstande die Wirkung der Glukose und Lävulose auf die Funktion des RES zu verstärken.

5) Deshalb ist zu vermuten, dass der Kongorotindex einen Anzeiger der Funktion des RES und die Prothrombinzahl einen Funktionszustand der Leberzellen darstellt.

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Experimentelle Studien über die Funktion des reticuloendothelialen Systems

III. Mitteilung

Einfluss des vegetativen Nervensystems auf die Funktion des RES und der Leber

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Seit Entdeckung des Zuckerzentrums in der Oblongata von Claude Bernard[1] ist es bekannt, dass die Leber durch die sympathischen und parasympathischen Nerven doppelt innerviert wird (Greving[2]). Ausser dem Zuckerzentrum fanden Matsuo und seine Schüler[3] das Vorhandensein des Ausscheidungszentrums von Farbstoff durch Leber im Schwanzteil des Nucleus vagi dorsalis in der Nähe des Zuckerzentrums in der Oblongata, und sie nannten dies Leberstich. Nach Matsuo[3] wird der Impuls des Zuckerstichs durch die sympathischen Nerven und der des Leberstichs durch die parasympathischen Nerven geführt. In Bezug auf das höhere Zentrum des vegetativen Nervensystems im Hypothalamus betont Kurotsu[4], dass es entlang des dritten Ventrikels gürtelartig wie a-parasympathische, b-sympathische und c-parasympathische Zone nebeneinanderliegt.

Wie in der II. Mitteilung berichtet, ist es schon begreiflich, dass der Kongorotindex die Funktion des RES und die Prothrombinzahl die der Leberzellen verraten kann. Deshalb ist in der vorliegenden Untersuchung zunächst genau zu erforschen, wie das vegetative Nervensystem beide Funktion beeinflusst. Die Auswahl der Versuchstiere sowie Methodik war die gleiche wie bei der letzten Mitteilung[5].

Versuchsergebnisse

1. Einfluss der vegetativen Nervengifte auf die Funktion des RES und der Leber

Wenn man Adrenalin in Menge von 0.5 mg pro kg unter die Haut

TABELLE I

Einfluss der peripheren vegetativen Nervengifte

		Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)		
				1	3	5
Adrenalin	K-index	1	58.7	66.8	81.7	65.6
		2	46.4	50.7	74.2	63.6
		Durchschnitt	52.3	58.0	77.6	64.6
		Differenz		2.5	21.0	8.6
	P-zahl	1	1.00	1.14	1.09	1.08
		2	1.00	1.09	1.08	1.07
		Durchschnitt	1.00	1.12	1.09	1.08
		Differenz		0.12	0.10	0.06
Neogynergen	K-index	1	62.5	68.3	72.2	61.1
		2	52.8	49.3	57.7	60.8
		Durchschnitt	57.8	59.8	65.3	57.8
		Differenz		− 9.8	−11.6	−17.5
	P-zahl	1	1.00	1.03	1.01	0.98
		2	1.00	1.00	1.01	1.03
		Durchschnitt	1.00	1.02	1.01	1.01
		Differenz		0.02	0.04	0.03
Pilocarpin	K-index	1	56.3	60.0	65.8	75.0
		2	54.7	45.7	43.3	70.0
		Durchschnitt	55.7	54.3	57.1	73.3
		Differenz		−15.2	−11.3	7.4
	P-zahl	1	1.00	0.99	1.01	0.98
		2	1.00	1.02	0.94	0.95
		Durchschnitt	1.00	1.01	0.98	0.97
		Differenz		0.01	−0.01	−0.04
Acetylcholin	K-index	1	55.2	58.7	44.9	57.7
		2	35.8	36.4	34.2	42.4
		Durchschnitt	46.3	48.2	39.9	50.0
		Differenz		− 1.3	−10.8	0
	P-zahl	1	1.00	1.00	1.03	0.99
		2	1.00	1.00	0.99	1.00
		Durchschnitt	1.00	1.00	1.01	1.00
		Differenz		0	0.02	−0.01
Atropin	K-index	1	50.0	71.4	80.0	75.2
		2	34.5	43.7	55.0	56.3
		Durchschnitt	41.8	59.2	69.5	68.8
		Differenz		14.2	23.4	23.3
	P-zahl	1	1.00	1.01	1.00	0.99
		2	1.00	1.02	1.01	1.00
		Durchschnitt	1.00	1.02	1.01	1.00
		Differenz		0.01	−0.02	−0.03

injiziert, so vermehren sich der Kongorotindex und die Prothrombinzahl, wie man in Tabelle I ersieht. Injiziert man dagegen Neogynergen in

Menge von 1 ccm, das 0.25 mg von sympathicuslähmendes Ergotamin enthält, pro kg unter die Haut, so vermindert sich der Kongorotindex in hohem Grad, aber die Prothrombinzahl neigt sich zu vermehren. Bei subkutaner Injektion des Pilocarpins wird eine Herabsetzung des Kongorotindex und der Prothrombinzahl hervorgerufen, doch verursacht Acetylcholin in Menge von 10 mg pro kg eine bedeutende Verminderung des Kongorotindex, während die Prothrombinzahl fast unbeeinflusst bleibt. Wenn man Atropin in Menge von 1 mg pro kg subkutan injiziert, so vermehrt sich der Kongorotindex auffallend, doch bleibt die Prothrombinzahl unverändert.

In Bezug auf das Blockierungsmittel der vegetativen Nerven sieht man aus Tabelle II, dass Imidalin (Benzylimidazolin), das von sympathicusblockierender Natur ist, in Menge von 10 mg pro kg eine mässige Verminderung des Kongorotindex und eine geringe Vermehrung der

TABELLE II

Einfluss der Blockierungsmittel der vegetativen Nerven

		Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)		
				1	3	5
Imidalin	K-index	1	41.0	53.5	53.3	50.0
		2	36.6	42.0	46.9	56.8
		Durchschnitt	38.8	48.0	50.3	47.8
		Differenz		– 4.6	– 1.2	– 1.2
	P-zahl	1	1.00	1.01	1.01	1.00
		2	1.00	1.05	1.03	0.99
		Durchschnitt	1.00	1.03	1.02	1.00
		Differenz		0.03	0.03	–0.02
Banthine	K-index	1	51.7	63.7	59.3	62.7
		2	49.0	50.0	56.8	59.5
		3	48.5	61.1	54.6	59.5
		Durchschnitt	49.7	58.3	56.7	62.3
		Differenz		5.4	2.7	8.9
	P-zahl	1	1.00	1.00	1.03	0.96
		2	1.00	1.00	0.98	0.94
		3	1.00	1.02	1.01	1.02
		Durchschnitt	1.00	1.01	1.01	0.97
		Differenz		0	–0.02	–0.06
Tonoplon	K-index	1	38.5	53.0	54.4	46.2
		2	27.5	39.2	37.5	31.4
		Durchschnitt	32.9	46.1	45.8	38.5
		Differenz		– 0.6	– 0.2	– 4.6
	P-zahl	1	1.00	0.90	0.94	1.02
		2	1.00	0.94	1.00	1.05
		Durchschnitt	1.00	0.92	0.97	1.03
		Differenz		–0.08	–0.02	0.01

Prothrombinzahl, wie Ergotamin, hervorruft. Dagegen verursacht das parasympathicusblockierende Banthine (Methanthelin) eine Steigerung des Kongorotindex und eine Herabsetzung der Prothrombinzahl, wie Atropin. Bei intramuskulärer Injektion des Tonoplons (Teträthylammoniums), das Blockierungsmittel der vegetativen Ganglien, in Menge von 30 mg pro kg konstatiert man eine Verminderung des Kongorotindex und der Prothrombinzahl.

Daraus erhellt, dass Sympathicusreizungsmittel die Funktion des RES herabsetzend und die der Leberzellen steigernd wirken, während parasympathicusreizende Gifte die Funktion des RES erhöhen.

2. Einfluss der Zentralnervengifte auf die Funktion des RES und der Leber

Wie man aus Tabelle III ersieht, vermindert sich der Kongorotindex und vermehrt sich die Prothrombinzahl nach subkutaner Injektion von Philopon (Pervitin), dem Stimulus der Grosshirnrinde, in Menge von 3 mg (1 ccm 0.3 % iger Lösung) pro kg, während bei intravenöser Injektion von Chloralhydrat, dem narkotischen Mittel der Grosshirnrinde, in Menge von 0.2 g (2 ccm 10 % iger Lösung) pro kg eine Steigerung des Kongorotindex und eine Verminderung der Prothrombinzahl verursacht werden. Wenn man 2 ccm 20 % iger Magnesiumsulfatlösung, des Stimulus vom Striatum, oder 1 ccm 0.1 % iger Pikrotoxinlösung, des Reizmittels vom sympathischen Zentrum, pro kg subkutan injiziert, so konstatiert man eine Steigerung des Kongorotindex, aber die Prothrombinzahl bleibt fast unverändert. Umgekehrt vermindert sich der Kongorotindex nach intravenöser Injektion des Vagostigmins (Prostigmins), des Stimulus vom parasympathischen Zentrum, in Menge von 5 mg pro kg.

In Bezug auf Luminal, das Hirnstammnarkotikum, konstatiert man eine Steigerung des Kongorotindex und eine Herabsetaung der Prothrombinzahl bei intramuskulärer Darreichung von 30 mg pro kg, eine Wirkung des Philopons auf beide Funktion wird also von Luminal nicht ganz völlig gehemmt.

Aus obigen Versuchsergebnissen ist zu vermuten, dass die Grosshirnrinde auf beide Funktion fördernd, aber das sympathische Zentrum hemmend und das parasympathische fördernd wirkt.

3. Einfluss der Punktion des vegetativen Nervenzentrums auf die Funktion des RES und der Leber

Um zu erfahren, welchen Einfluss das vegetativen Nervenzentrum auf den Kongorotindex und die Prothrombinzahl ausübt, punktierten wir nach Kurotsu'scher Methode[4]. Wenn man das sympathische Zentrum punktiert, so steigen, wie Tabelle IV zeigt, der Kongorotindex und die Pro-

Tabelle III

Einfluss der Zentralnervengifte

		Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)		
				1	3	5
Philopon	K-index	1	37.0	38.1	36.8	58.6
		2	34.4	43.7	38.5	50.0
		Durchschnitt	35.0	41.0	37.6	54.6
		Differenz		– 5.8	–16.5	2.1
	P-zahl	1	1.00	1.02	1.04	1.03
		2	1.00	1.00	1.02	0.99
		Durchschnitt	1.00	1.01	1.03	1.01
		Differenz		0.01	0.04	0
Chloralhydrat	K-index	1	41.8	52.8	56.8	72.5
		2	29.7	71.6	54.4	56.5
		Durchschnitt	32.2	61.1	55.5	63.7
		Differenz		17.1	3.8	14.0
	P-zahl	1	1.00	1.00	0.92	0.98
		2	1.00	1.00	0.90	1.00
		Durchschnitt	1.00	1.00	0.91	0.99
		Differenz		0	–0.08	–0.02
Magnesium-sulfat	K-index	1	44.3	59.0	69.7	50.0
		2	42.1	62.8	65.4	64.5
		3	37.9	45.9	48.9	50.0
		Durchschnitt	41.7	54.7	61.9	53.5
		Differenz		8.5	9.1	– 4.7
	P-zahl	1	1.00	1.03	1.04	0.97
		2	1.00	0.99	1.07	1.08
		3	1.00	1.01	1.01	0.94
		Durchschnitt	1.00	1.01	1.04	1.00
		Differenz		0	0.02	–0.04
Pikrotoxin	K-index	1	46.4	50.0	44.7	56.9
		2	43.2	55.0	65.8	62.5
		Durchschnitt	44.7	52.3	56.1	60.4
		Differenz		4.4	7.1	12.0
	P-zahl	1	1.00	0.97	0.99	1.03
		2	1.00	1.01	1.04	1.10
		Durchschnitt	1.00	0.99	1.02	1.06
		Differenz		–0.02	–0.01	0.03
Vagostigmin	K-index	1	70.7	50.0	69.2	60.0
		2	46.6	47.3	53.7	53.7
		Durchschnitt	59.6	48.9	60.5	56.8
		Differenz		–13.9	– 3.2	– 6.5
	P-zahl	1	1.00	1.00	0.98	1.00
		2	1.00	1.00	1.03	1.03
		Durchschnitt	1.00	1.00	1.01	1.02
		Differenz		–0.01	–0.02	–0.01
Luminal	K-index	1	50.0	55.6	68.0	65.1
		2	45.8	70.6	66.7	65.0
		Durchschnitt	47.8	63.0	67.3	65.5
		Differenz		13.0	15.2	14.0
	P-zahl	1	1.00	1.00	1.00	0.96
		2	1.00	1.00	0.99	0.92
		Durchschnitt	1.00	1.00	1.00	0.94
		Differenz		0	0.01	–0.07
Philopon nach Luminal	K-index	1	55.3	67.9	75.0	73.8
		2	50.0	60.5	55.7	60.2
		Durchschnitt	52.6	63.8	64.4	66.5
		Differenz		– 0.6	– 7.3	– 3.6
	P-zahl	1	1.00	1.01	1.02	1.02
		2	1.00	1.01	1.02	1.02
		Durchschnitt	1.00	1.01	1.03	1.02
		Differenz		0.01	0.04	0.01

TABELLE IV

Einfluss der Punktion des vegetativen Nervenzentrums

Punktionsstelle		Nr. des Kaninchens	Vor Injektion	Zeit nach Injektion (St.)			
				3	5	8	12
Sympathisches Zentrum	K-index	1	56.4	73.3	65.2	74.2	58.0
		2	54.3	57.2	84.8	64.4	53.1
		Durchschnitt	55.4	65.3	75.0	69.3	55.6
		Differenz		8.8	13.3	13.0	0.5
	P-zahl	1	1.00	1.07	1.01	1.03	1.01
		2	1.00	1.06	1.03	1.01	1.03
		Durchschnitt	1.00	1.07	1.02	1.02	1.02
		Differenz		0.05	0.01	−0.03	−0.03
Parasympathisches Zentrum	K-index	1	64.5	38.1	41.8	43.3	54.4
		2	51.7	44.6	42.7	41.9	42.0
		Durchschnitt	58.4	41.4	42.2	44.4	48.3
		Differenz		−17.4	−20.6	−14.0	− 8.9
	P-zahl	1	1.00	1.06	0.97	1.00	1.01
		2	1.00	0.96	0.96	0.95	1.00
		Durchschnitt	1.00	1.01	0.97	0.98	1.01
		Differenz		−0.01	−0.04	−0.07	−0.04

thrombinzahl auf, in ähnlicher Weise wie bei Adrenalin. Punktiert man das parasympathische Zentrum, so vermindert sich der Kongorotindex und die Prothrombinzahl wie bei Pilocarpin.

Daraus könnte man sehr wahrscheinlich schliessen, dass die Reizung des sympathischen Zentrums eine Herabsetzung der Funktion des RES mit Steigerung der Funktion der Leberzellen, aber die des parasympathischen Zentrums eine Steigerung der Funktion des RES mit Herabsetzung der Funktion der Leberzellen verursacht.

DISKUSSION

Wie schon in der II. Mitteilung erwähnt, ist es anzunehmen, dass man unter sogenannter Leberfunktion die Summe des epithelialen und des vaskulären Parenchyms verstehen kann. Doch ist es noch nicht ganz kalr, wie das vegetative Nervensystem beide Funktion der Leber beeinflusst. In Bezug auf Förderungsnerven der Gallensekretionsfunktion der Leber bestehen Eiger[6], Matsuo und seine Schüler[3] auf Parasympathicus, im Gegensatz dazu Kurotsu und seine Schüler[4] auf Sympathicus. Nach Matsuo[3] wird die Farbstoffausscheidungsfunktion der Leber von parasympathischen Nerven befördert, aber Haskovec[7] bericht, dass die Kongorotausscheidungsfunktion von Sympathicus fördernd und von Parasympathicus hemmend beeinflusst wird.

Weiterhin wird eine Steigerung des Prothrombins durch Adrenalin-

injektion von Tocantins und O'Neil[8] beobachtet.

Betreffs der phagozytären Fähigkeit der Sternzellen betonen einige Autoren wie Händel und Malet[9] eine Herabsetzung durch Adrenalin, doch berichten Letterer und Bogendörfer[10] über Unveränderlichkeit durch Sympathicusabschneidung.

Fassen wir unsere Ergebnisse zusammen, so ist es klar, dass der Einfluss des vegetativen Nervensystems auf den Kongorotindex und die Prothrombinzahl ganz verschieden ist, indem das sympathicusreizende Mittel eine Vermehrung des Kongorotindex und der Prothrombinzahl und das parasympathicusreizende Mittel eine Verminderung des Kongorotindex herbeiführen, sei es peripheral oder sei es zentral wirkend. Die obenerwähnten Resultate werden auch bei Reizung des vegetativen Nervenzentrums durch Punktion beobachtet. Daraus folgt, dass Sympathicus auf Funktion der Leberzellen fördernd und auf diejenige des RES einschliesslich der Sternzellen hemmend, dagegen Parasympathicus auf die ersteren hemmend und auf die letzteren fördernd einwrikt.

Ausserdem beobachteten Kurotsu und seine Schüler[4] eine Hyperglykämie nach der Punktion des Sympathicuszentrums und eine Hypoglykämie nach derjenigen des Parasympathicuszentrums. Weiterhin berichtet Musha[11] aus unserem Institut, dass Glykogenverarmung und Hemmung der Glykogenoneogenesis in der Leber nach Punktion des sympathischen Zentrums und Glykogenvermehrung und Steigerung der Glykogenoneogenesis nach derjenigen des parasympathischen Zentrums hervorgerufen werden. Deshalb ist es begreiflich, dass Zuckerstich von Claude Bernard[1] der Punktion des sympathischen Zentrums und Leberstich von Matsuo[3] derjenigen des parasympathischen Zentrums entspricht.

Zusammenfassung

Aus obenerwähnten Ergebnissen können wir folgenderweise schliessen:

1) Von den peripheren vegetativen Nervengiften verursachen Adrenalin sowie Atropin eine Vermehrung des Kongorotindex bei Darreichung davon, dagegen Pilocarpin, Acetylcholin sowie Neogynergen eine Verminderung desjenigen. Im Gegensatz dazu steigt die Prothrombinzahl durch Adrenalin auf, aber fällt durch Pilocarpin herab, während diejenige fast unverändert bleiben bei anderen Nervengiften.

2) In Bezug auf Blockierungsmittel der vegetativen Nerven ruft Imidalin (Benzylimidazolin) eine Herabsetzung des Kongorotindex mit Steigerung der Prothrombinzahl hervor, dagegen Banthine (Methanthelin) eine Vermehrung des ersteren mit Verminderung des letzteren, während bei Tonoplon (Tetraäthylammonium) der Kongorotindex sowie Prothrombinzahl sich vermindert.

3) Betreffs der Zentralnervengifte finden wir eine Verminderung des Kongorotindex durch Philopon (Pervitin) sowie Vagostigmin (Prostigmin), dagegen eine Vermehrung desjenigen durch Chloralhydrat, Magnesiumsulfat, Pikrotoxin sowie Luminal. Aber die Prothrombinzahl vermehrt sich durch Philopon (Pervitin), vermindert sich dagegen durch Chloralhydrat sowie Luminal, während diejenigen durch andere Gifte unverändert bleiben. Ausserdem wird die Wirkung des Philopons (Pervitins) durch Luminal nicht ganz völlig gehemmt.

4) Durch Punktion des Sympathicuszentrums nach Kurotsu'scher Methode wird eine Steigerung des Kongorotindex und der Prothrombinzahl hervorgerufen, dagegen eine Herabsetzung der beiden durch diejenige des parasympathischen Zentrums.

5) Daraus folgt, dass Sympathicus auf die Funktion der Leberzellen fördernd und auf diejenige des RES einschliesslich der Sternzellen hemmend, aber Parasympathicus auf die erstere hemmend und auf die letztere fördernd einwirkt.

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Cytodiagnosis of Malign Tumors by Means of the Needle Biopsy and Smear Method

Part 2. Cytological Differences between Carcinoma and Sarcoma

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As described in the preceding report,[1] the writer and his co-worker carried out cytological observations of smear preparations, comparing these with tissue sections. From the materials obtained by needle biopsy some smear preparations were made, and what remained of the materials were made into tissue sections. Smears could be observed about half an hour after the biopsy, and the findings were compared with those of tissue sections which were ready one or two days later. While decisive diagnoses were given histologically, the more experienced the technic of preparing and observing smears, the more accurate was the diagnosis by smears. And it became possible to be asserted that in the majority of cases the tissue was distinguished either as malign or benign, and either epithelial or non-epithelial, in smear preparations.

Reports available about smears of malign tumors are abundant, but they are mostly descriptions about cancers, while investigations of sarcomas or of differentiations between carcinoma and sarcoma are not yet fully proved. In this paper are discussed the cytological features, in order to distinguish between carcinoma and sarcoma.

Experimental

Materials and Methods

The methods are the same as in the preceding report[1].

The cases which provided both histological and cytological preparations amounted to 150. Among them the cases of carcinomas were 63, and those of sarcomas 17, others being non-malignant tissues. The classification of cases are shown in Tables I and II.

As non-malignant tissues there were those of acute and chronic inflammation, lymphadenitis simplex, tuberculous lymphadenitis, granu-

TABLE I
Classification by Punctured Region

	Carcinoma	Sarcoma	Non-malignant Tissue
Lymph node	18	7	10
Liver	26	0	37
Head	0	2	2
Neck	2	1	0
Breast	8	1	1
Abdomen	7	2	13
Prostatic tumors	2	2	2
Limbs	0	2	3
Total	63	17	70

TABLE II
Classification of Malignant Tumors

Carcinoma	Number of cases	Sarcoma	Number of cases
Gastric carcinoma	20	Small round cell sarcoma	2
Cholangioma	9	Large round cell sarcoma	3
Hepatoma	10	Short spindle cell sarcoma	2
Pancreatic carcinoma	4	Spindle cell sarcoma	3
Thyroid carcinoma	3	Polymorphcellular sarcoma	2
Prostatic carcinoma	3	Lymphosarcoma	1
Pulmonary carcinoma	3	Reticulosarcoma	3
Mammary carcinoma	3	Meningeoma sarcomatosum	1
Hypernephroma	2	Total	17
Carcinoma of colon	1		
Uterine carcinoma	1		
Carcinoma of thymus	2		
Carcinoma of gallbladder	1		
Cancerous endothelioma	1		
Total	63		

lation tissues, necrotic tissues, muscle tissues, fat tissues, fibromas, cysts, tissues of various glands, and liver tissues with various diseases.

Observations

1. Features of carcinoma cells

Combination of cells. In carcinoma cases cells are very often seen partly as cell combinations and sometimes arranged in so-called rosettes. It is considered that the fact shows the character in the cells of combining

with each others, and this character is found more or less in all the carcinoma cases. In the preparations furnished by this method it can be observed how the process of aspiration and smearing scatters the cells or cell combinations in the slide, so that the degree of combination power of cells can be surmised. Generally speaking, in the cases in which cells are varied, having large nuclei, as obvious features of malignancy, the combination is loose, while in the cases in which cells are small and similar, it is very often compact. That shows malignancy of cells tends to weaken the combination of cells with each other, while in all carcinoma cases it is more or less recognizable. For example, in the cases of a pancreatic carcinoma (Fig. 2D), a carcinoma of cervix of uterus, and a gastric carcinoma, the cell combination is considerably close, and in the cases of hepatoma it is relatively loose in general. In the case of gastric cancer (Fig. 2E), it is very loose, a majority of cells being scattered individually by the process of aspiration and smearing, though it is obviously recognized with the rest. Histologically this case reveals to a certain extent similarity to reticulosarcoma, and clinically makes many metastases into various parts of the body. Weakness of cell combination seems to constitute a factor in the tendency to metastasis. The cases giving rosettes are not many, but these are considered to be remaining polarity.

Nuclei of carcinoma cells are generally large, many of them being 12 to 25 μ in diamter and some amounting to 30 to 40 μ, while giant nuclei larger than them are also to be found. Among these can be found smaller ones from 5 to 7 μ in diameter, so a variety in size is very obvious. But in some cases relatively small and similar nuclei are seen. Their shapes are mostly irregular, round or oval, but some cells resemble a kidney or are grotesquely complicated in shape. Moreover they are sometimes observed so crushed and transformed, that they vary greatly in shape. The boundaries of nuclei are mostly not smooth.

Nuclear structure is reticular or granular, and rather fine, but more coarse than that of myeloblast or promyelocyte. Chromatin is generally plentiful and stained deeply. Chromatin strands are very compact, but their boundaries are very distinct. They somewhat resemble half-matured megaloblasts. Nuclear structure considerably varies according to cases and also to maturation of cells even in a single case. The structure is obvious in middle-sized nuclei, and small nuclei are stained very deeply and evenly.

Nucleoli. Hauptmann[2] exemplified several types of nucleoli of carcinoma cells, but in the present cases they very often stain bluish, though sometimes seen not stained or light-colored in chromatin mass. They vary in number, size, and shape. Giemsa staining reveals nucleoli not so conspicuous as Mann's methylblue-eosin staining does, but the

former is efficient for distinguishing cells.

Nucleoli of carcinoma cells are often very large and sometimes larger than erythrocyte, so that nucleolar-nuclear ratio increases, reaching not rarely to 0.5 or more than that. In the present cases large nucleoli are not seen with all the cancer cases, and in the cases observed do not exist in all the cells, though especially large nucleoli are valuable for distinguishing cancer cells. Their number also sometimes increases and in some cases a nuclei possesses more than ten nucleoli. Moreover, in two cases extrusion of nucleolus from nucleus is observed. Owing to a moderate degeneration of cells a nucleus loses its cytoplasm and transforms its shape, nucleolus moves towards the periphery of nucleus and projects, and, when nucleus is destroyed, nucleolus is put out (Fig. 1).

Fig. 1. Nucleolus-extrusion
N: nucleus, n: nucleolus, E: erythrocyte

This is due to the fact that resistance of nucleoli is stronger than that of nuclei. Besides, in some cases, as Tischendorf[3] said, when at mitosis the formation of chromosomes is seen, bluish nucleoli are still distinctly observed (Fig. 2H).

Cytoplasm of carcinoma cells is not well developed and not plentiful, though in some cases considerably plentiful cytoplasm is seen. In cell combination border line of each cell is sometimes not apparent, and many nuclei are arranged closely in a common cytoplasmic area crammed with them. Sometimes fine azurophilic granules or round azurophilic corpuscles of various sizes are found in cytoplasm. Moreover, in some cases vacuoles are seen, and among them two sorts are recognized. One means degenerative vacuolization, and the other is obviously not due to degeneration. These vacuoles are seen alone in a single cell and changing their form growing in size, and in them violet-stained substance is recognized, which in some cases reveals mucicarmin staining positive. This vacuolization means secretory function still remaining in cancer cells which grow from a secretory organ. In some cases on one or two poles of the cells reddish-stained substance is seen.

Mitosis. Even in malign cells mitotic figures are usually not very evident, but in some cases they are very obviously seen, and in cases they are not, mitoses are mostly found, if carefully observed. Atypical mitotic figures, which are sometimes regarded as one of the features of malignancy,

for example, those with three poles or a few chromosomes, are seldom found. Amitotic or direct division of nuclei is often seen.

Giant cells. In the cancer are often found giant cells, which are distinguished into two groups of giant cells with giant nuclei and polynuclear giant cells.

Degeneration. In the case of severe degeneration, tissue has been reduced into debris and can no longer be observed as cells in smear. In the case of slight degeneration vacuolization is found in cytoplasm, or nuclei lose their cytoplasm and remain as naked nuclei. Mild degeneration takes place often in the tissue which has histologically good blood circulation.

Polymorphism. A great variety of cancer cells are found according to cases, and, moreover, even in a single case cells and especially nuclei are very various in shape and size. Large forms of nuclei are about two and a half times as large as small ones. Besides, the shape is various, staining is either deep or light, and mitosis and amitosis are seen. Nucleoli are also various in shape and size. Cytoplasm is various in size and sometimes having vacuoles, and two or three nuclear cells are found. As these cells are arranged in groups or in cell combinations, they are apparently in special cellular features. The polymorphism is more distinct in carcinoma simplex (Fig. 2A) than in adenocarcinoma.

Kind specificity. In smears kind specificity of various carcinomas is not obvious generally. In the cases of prostatic cancers and cancers o-thyroid gland, nuclei are not so large and their polymorphism not apf parent, but these are only insufficient characteristics of malignancy. In some cases of gastric cancer is seen reddish-stained substance on one or two poles of cells, or secretory vacuoles in cytoplasm. The characteristic features of hepatomas are described in the preceding report.

2. Features of sarcoma cells

Arrangement of cells. In cases of sarcoma, tumor cells combined with each other are not generally recognized, and they are scattered individually and irregularly. Cells of some reticulosarcomas have processes and conjugate partly to each other (Fig. 2O). But direct face-to-face combination between cells is never recognized. In a case of meningeoma sarcomatosum can be recognized cell combination. This is, however, an exceptional case due to the tissue from which the tumor grows. In this tumor the relation between tumor cells and capillaries corresponds to that in sarcoma. That is, in the case of tender sarcoma, when tissue is smeared by pressing, capillaries remain undestroyed because of their resistance, and along and about them tumor cells are arranged (Figs. 2I, 2J and 2R).

Nuclei are 10 μ to more than 20 μ large in diameter. Many of their

shapes are irregular, round or oval, but their border line is relatively smooth. Nuclear structure is not so distinct and finer than that of carcinoma, and boundaries of chromatin nets are less distinct. Nucleoli also are mostly not distinct. Because of poor cytoplasm nuclear-cytoplasmic ratio is very large. A small number of mitosis are found, and atypical mitoses with very few chromosomes are present (Fig. 2M). In a case of very immature small round cell sarcoma, numerous mitoses are found (Fig. 2K).

Cytoplasm of sarcoma cells is poor. In cases of large round cell sarcoma and long spindle cell sarcoma cytoplasm is relatively rich, but poorer than that in the case of carcinoma with larger cytoplasm. The shape of cytoplasm corresponds to the shape by which the tumor is classified histologically, that is, small and large round cells, short and long spindle cells and giant cells respectively show their characteristic figures. However, polymorphism of each cell considerable in shape and size is found. In cases of reticulosarcoma are seen various cells. In some cases are seen round cells, and in one case many cells have short processes (Fig. 2N), and in another case some cells have spindle-shaped cytoplasm which is communicated in part with each other apparently (Fig. 2O). In a case of polymorphcellular sarcoma bluish tone of immature cytoplasm fades as it grows at the circumference of nuclei, and here then appear azurophilic granules. In this case each nucleus is relatively similar, while a cell has one to more than thirty nuclei, large one amounting to 200 μ in diameter (Fig. 2Q). Polynuclear giant cells are seen also in spindle cell sarcoma.

As classification of sarcoma is by types of the cells, it is more distinct with smear preparations than with section tissues.

Discussion

The study on diagnosing tumors with smear preparations has been continued by enthusiastic investigators for the last 25 years. Since the works of Papanicolaou and his followers, it has received attention of many practitioners, and been partly applied for practical purposes. But the cytological features of malign cells, often described in literatures, are mostly those of cancerous tumor cells. Descriptions about sarcoma or about differences between carcinoma and sarcoma are not many. Only Tischendorf[3] or Dudgeon and Barrett[4] have referred to them.

According to the writer's observations, cancerous tumor cells have the features of both epithelial cells and malign cells, while sarcomous tumor cells do not have the features of epithelial cells, but have the cell shape peculiar to each sort of sarcomas and features of malignant cell less distinct than those of cancer cell. As for the features of epithelial cell, the combination of cells with one another can be first pointed out.

Though malignancy of cells tends to weaken the combination of epithelial cells, yet it is always more or less recognizable in all of the present cases without exception, and sometimes cells are found arranged in rosettes. The nuclear structure of cancer cells is rather coarse, boundaries of chromatin strands are distinctly seen, nucleoli are often large, distinct and stained bluish, and, where degeneration of nuclei exists, nucleoli-extrusion is sometimes seen. Cytoplasm of larger-sized cancer cells is more plentiful than that of sarcoma cells, and in some cases secretory vacuoles in it, and also in some other cases reddish-stained substance around it, are found. In the case of small cell cancer each of these cytological features is sometimes not obvious, and in these cases the cells with poor cytoplasm are combined closely, and their nuclei are densely arranged and partly transformed their shape by pressing each other.

In the case of sarcoma face-to-face combination of cells is not recognized, cells are arranged individually and irregularly, and, if capillaries are found, sarcoma cells are seen about and along them. While being polymorphous, cells show their own peculiar shapes by which sarcomas are classified histologically, that is, they are small or large round cells, short or long spindle cells, or polynuclear giant cells.

Nuclei are almost round in round cells, and oval in spindle cells. Their structure is not so obvious as that of cancer cells, and their polymorphism is not distinct as compared with the latter's even in polymorphcellular sarcoma. Nucleoli are also not apparent. Mitosis is not usually frequent, except for a case of immature small round cell sarcoma.

As above described, by following the writer's method, differences between carcinoma and sarcoma in cytological features are considerably obvious. So that after some experience it is relatively easy to distinguish carcinoma and sarcoma in smears. Moreover, it is important, when one diagnoses malignancy of cells in smears, to have it always in mind whether they are epithelial or non-epithelial.

Summary and Conclusions

1. With 150 cases needle biopsy was carried out and from the materials obtained by biopsy several smears and tissue sections were prepared.

2. In the smears cytological features of malignant cells, especially differences between carcinoma and sarcoma were observed. The findings were compared with those in tissue sections, and the diagnoses were confirmed.

3. By following the writer's method, differences between carcinoma and sarcoma in cytological features were considerably obvious, so that, after some experience, it became relatively easy to distinguish carcinoma

and sarcoma in smears.

4. To diagnose malignancy of cells by this method, it is important to have it always in mind whether they are epithelial or non-epithelial.

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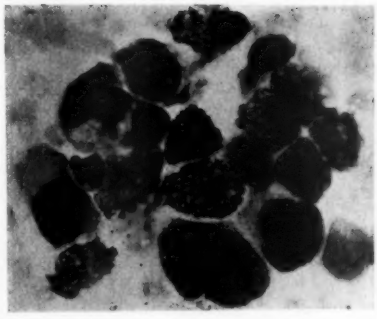
2) Hauptmann, Amer. J. Path., 1948, **24**, 1199.
3) Tischendorf, Klin. Wschr., 1941, 398.
4) Dudgeon & Barrett, Brit. J. Surg., 1934–35, **22**, 4.

Fig. 2

A



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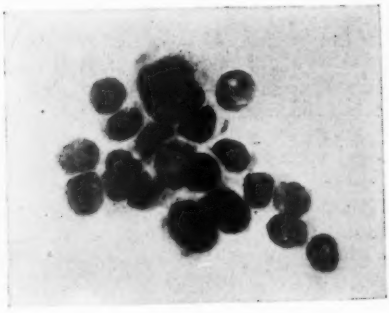

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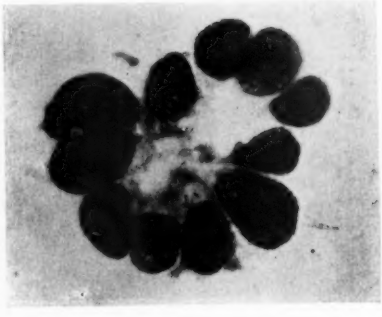

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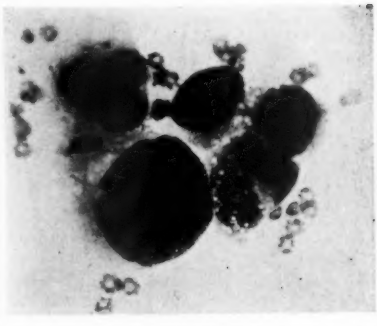

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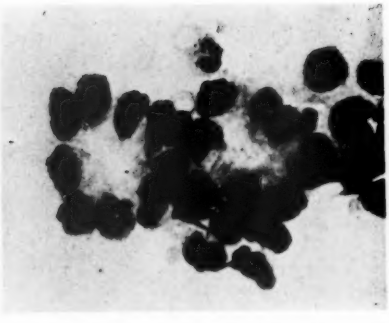

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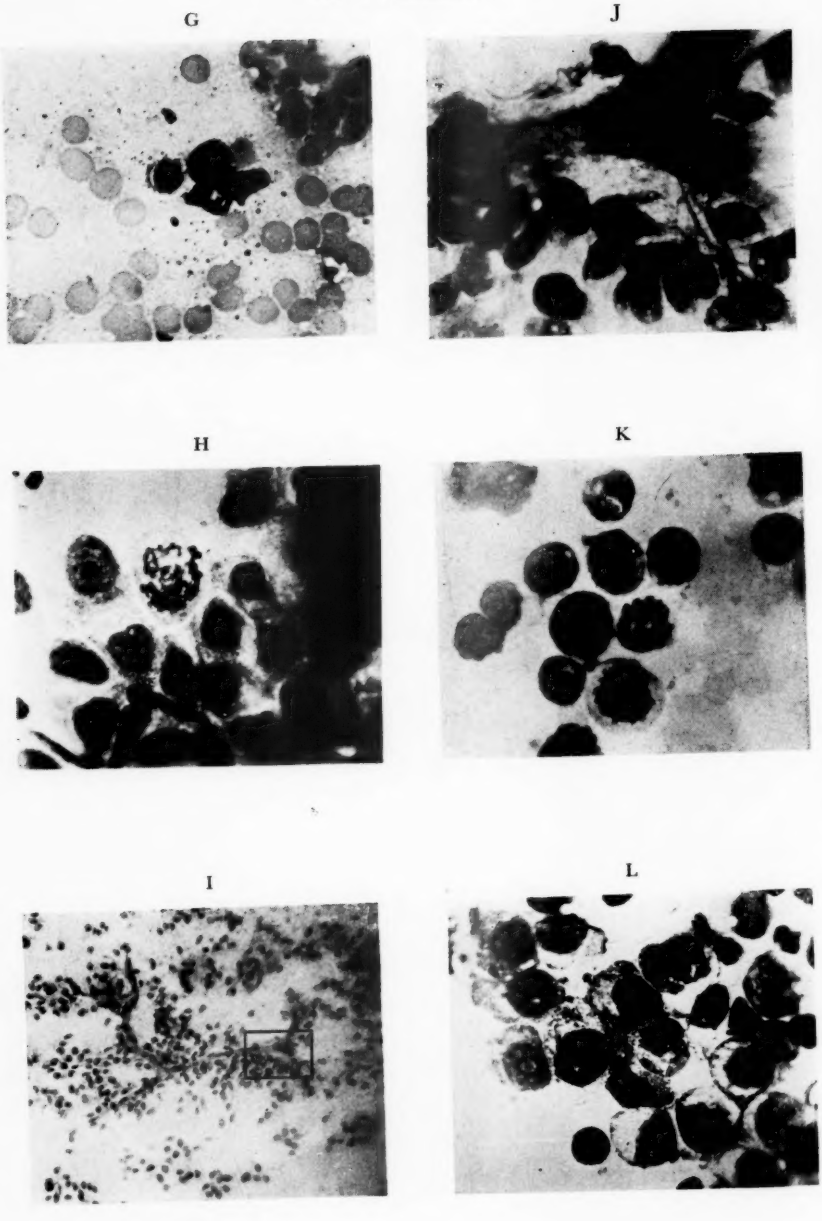

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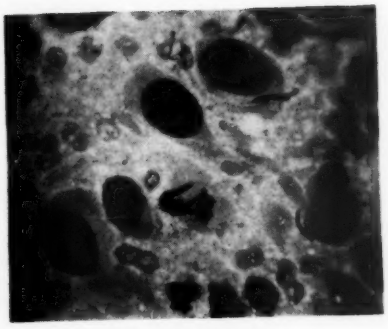

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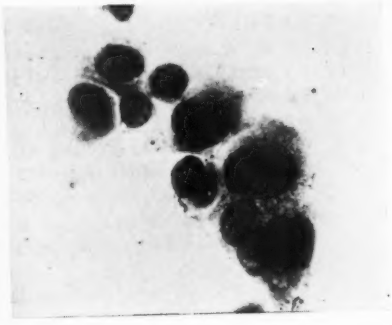

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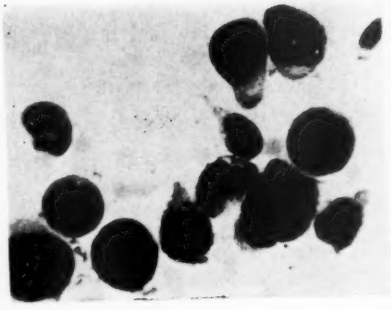

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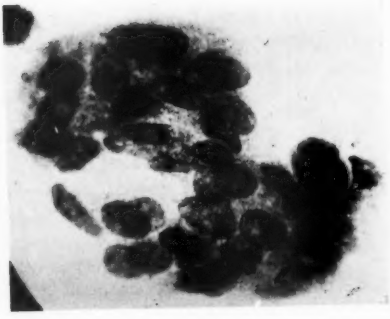

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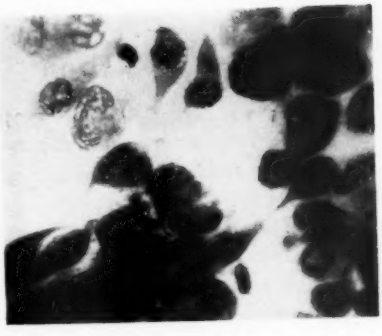

R

Description of microphotograms (Fig. 2)

A. Cell combination of carcinoma cells with polymorphism. Case: supraclavicular lymph node metastasis in gastric carcinoma, carcinoma simplex.

B. A rosette of carcinoma cells. Case: ditto.

C. Cancerous giant nucleus and giant nucleoli, the latter being unstained and showing as large as erythorocyte. Case: supraclavicular lymph node metastasis in gastric carcinoma, carcinoma simplex.

D. Combination of cells is close, and bluish-stained nucleoli are obvious. Case: carcinoma of pancreas, adenocarcinoma.

E. Individually scattered three carcinoma cells, large bluish-stained nucleoli as large as erythrocyte, and secretory vacuolisation in cytoplasm. Case: supraclavicular lymph node metastasis in gastric carcinoma, carcinoma simplex.

F. Two rosettes of small cancer cells with little variety. Characteristics of malignancy is not obvious, but those of epithelial cells are distinct. Because of needle biopsy of lymph node, these epithelial cells reveal metastasis of malignant cells. Case: supraclavicular lymph node metastasis in prostatic carcinoma, adenocarcinoma.

G. Nucleolus-extrusion. n: large nucleolus extruded, N: degenerated and destroyed nucleus. Case: supraclavicular lymph node metastasis in gastric carcinoma, carcinoma simplex.

H. In mitotic phase bluish-stained nucleolus is distinctly present.

I. Sarcoma cells and capillaries. Spindle cell sarcoma (Lower enlargement).

J. Ditto (Oil immersion).

K. Small round cell sarcoma, two cells in mitosis.

L. Large round cell sarcoma.

M. Osteosarcoma, spindle cells with variety in shape, atypical mitosis with a small number of chromosomes.

N. Reticulosarcoma, some cells having short processes.

O. Reticulosarcoma, cells being in part communicated with cytoplasmic processes.

P. Polymorphcellular sarcoma.

Q. Ditto, polynuclear giant cells.

R. Meningeoma sarcomatosum (Lower enlargement).

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Studies on the Defense Mechanism of the Body and the Neurohumoral Regulation

Report IV. Further Studies on the Neurohumoral Regulation of the Adrenal Cortex

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In the III. report we[1] have already reported that the stimulation of the N. splanchnicus caused a remarkable increase of the chemocorticoid substance in the adrenal venous blood only on the stimulated side and given our opinion in respect to the possibility that the secretory mechanism of the adrenal cortex might be regulated not only by the humoral, but also by the neural control.

In this paper we are going to report the results obtained from our further experimental studies concerning this problem.

Experimental

The Effect of the Stimulation of the N. Splanchnicus on the Chemocorticoid Content in the Adrenal Venous Blood of Hypophysectomized Dog

We extirpated pituitary glands on five dogs beforehand. The postmortem examination revealed that the pituitary glands had been extirpated completely in two dogs and partially in three dogs (one half of the gland extirpated in two cases and one-third in one.)

From one to five days after the extirpation of the pituitary gland we stimulated the N. splanchnicus on the left side by the same method mentioned in the former III. report.

The method of extraction and determination of the chemocorticoid substance in adrenal venous blood was given also in the III. report (modified Heard-Sobel's method).

The result of this series of experiment is given in the following Table I.

TABLE I

Chemocorticoid Content in the Adrenal Vein, Effect of N. Splanchnicus Stimulation in Hypophysectomized Dogs

(γ per 10 cc. adrenal venous blood)

Time (min.)	0	5	10	15	20	25	30	35	40	45	60	Rate of increase
Exp. 1. Hypophysis partially extirpated.	53			60		345						+292
Exp. 2. Hypophysis partially extirpated.	138	147			180		288					+150
Exp. 3. Hypophysis partially extirpated.	90		258		175		120		110			+168
Exp. 5. Hypophysis totally extirpated.	100		177		400							+300
Exp. 5. Hypophysis totally extirpated.	80		115		145		160			145	80	+ 80

As this Table I indicates, the chemocorticoid content in the adrenal venous blood on the stimulated side is remarkably increased by the splanchnic nerve stimulation, amounting to the maximum between 5–30 minutes after the end of the stimulation.

This result is quite similar with that obtained from the nonhypophysectomized cases (p. 157 in the III. report). Only there is a slight tendency that the chemocorticoid content reaches its maximum a little more slowly in the hypophysectomized dogs (compare with the table in page 157 of the III. report).

The Histological Studies of the Adrenal Cortex in the Case of the Stimulation of the N. splanchnicus

Immediately after the end of the above experiment, the adrenal glands were examined histologically by the Sudan III staining comparatively on the stimulated, non-stimulated side and also adrenal of the normal untreated animal. And marked difference was confirmed in histological features between both sides as follows, the sudanophilic lipid granules in the adrenal cortex on the stimulated side showed a remarkable change, they are clearly decreased in number in all zones, become fine granular, and thus these zones appear brightly, especially noticeably in the reticular and fascicular zones.

The so-called transitional zone between the glomerular and fascicular zones (sudanophobe zone) (Bennett[2] 1939), which is regarded as the presecretory zone and appear brightly in the normal condition owing to its poor sudanophilic granules, was filled with fine lipid granules and therefore became undiscriminative.

This histological feature corresponds just to the state of the elevated function of the adrenal cortex, as has already been pointed out by Selye,[3] Deane[4] and other authors (Fig. 1). The sudanophilic granules in the adrenal cortex of the non-stimulated side are gross and numerous and the transitional zone was clearly visible on the contrary, suggesting rest and accumulation of the cortical activity. (Fig. 2).

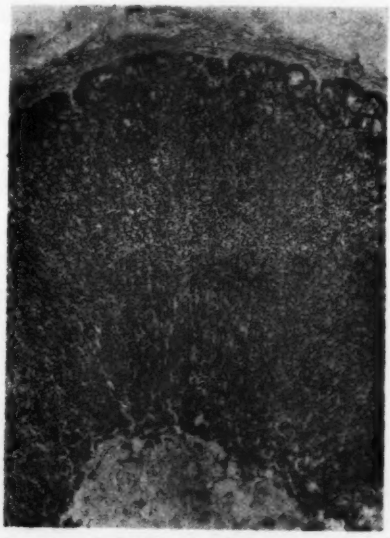

Fig. 1. Adrenal cortex of the N. splanchnicus-stimulated side. Sudan III ×50 (dog)

In comparison with the untreated normal dog, the histological features of the adrenal cortex on the non-stimulated control side of the experimented dog showed a small difference: the most outside layer, the zona glomerulosa, of the non-stimulated as well as of the stimulated side contained lipid granules relatively abundantly as compared with that layer of the untreated normal dog.

We also examined the adrenal cortex histologically in the rabbit whose one side splanchnic nerve had been sectioned thirty days previously. In this case the lipid granules of the cortical layers on the sectioned side

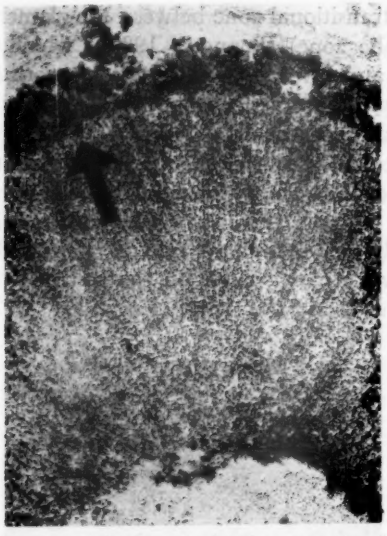

Fig. 2. Adrenal cortex of the non-stimulated control side. Sudan III staining ×50 (dog)

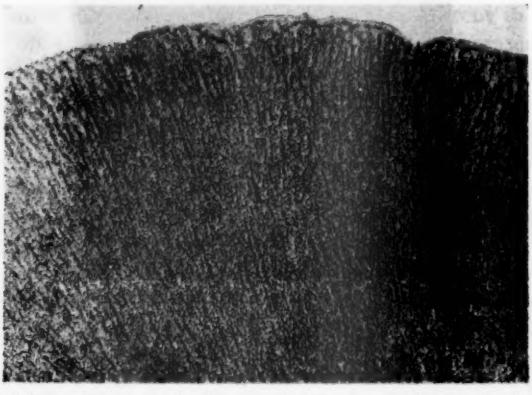

Fig. 3. Adrenal cortex of the N. splanchnicus sectioned side. Sudan III staining ×50 (rabbit)

showed features just as the type of rest and accumulation as compared with the control side, suggesting the reduced secretory activity of the cortical hormones. (Figs. 3 and 4).

In the previous report (III. report) we have published that the stimulation of the N. splanchnicus caused a remarkable increase of the

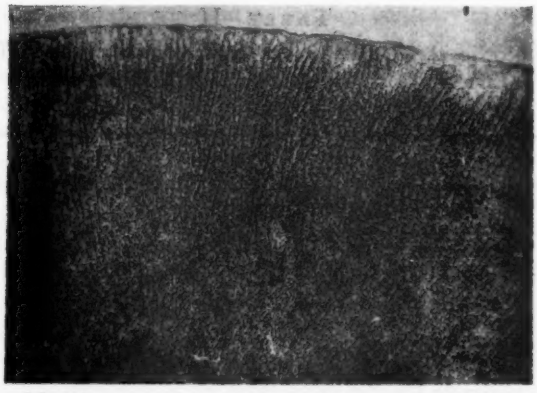

Fig. 4. Adrenal cortex of the N. splanchnicus non-sectioned control side ×50 (rabbit)

chemocorticoid substance in the adrenal vein blood on the stimulated side, and suggested that there might be some neural or neurohumoral control in respect to the secretory mechanism of the adrenal cortex, besides well known humoral control through the anterior pituitary gland. The above experimental results give a further important knowledge, indicating that the increased chemocorticoid substance in the adrenal venous blood confirmed in the time of stimulation of the N. splanchnicus might be evoked without the existence of the pituitary gland. In this paper we could confirm furthermore the histological appearance of the adrenal cortex after the stimulation of the N. splanchnicus suggesting the increased activity of cells in the cortical layers, just corresponding to the increase of the chemocorticoid substance in the adrenal venous blood.

On the Influence of the Epinephrine-injection upon the Chemocorticoid Substance in the Adrenal Venous Blood

In this set of experiments the influence of the intravenous injection of epinephrine on the chemocorticoid output in the adrenal venous blood was studied on the normal as well as on the hypophysectomized dogs.

A. Experiments on the normal dogs

0.35–0.5 cc. of 0.1% epinephrine-solution was injected into the femoral vein at one time and the chemocorticoid in the adrenal venous blood was estimated several times between 5 and 60 minutes after the injection.

The result is given in the Table II.

As the Table shows, the chemocorticoid content was increased from 585 γ/10 cc. blood to 933 γ in fourty five minutes (No. 1), from 200 γ

TABLE II

Chemocorticoid Content in the Left Adrenal Vein in Dogs.
Effect of Intravenous Injection of Epinephrine

(γ per 10 cc. blood)

Exp. No.	Time (min.) / Method of Injection.	0	5	10	15	20	30	40	45	60	Rate of Increase
1.	0.5 cc. 0.1% Epineph. inj. intrav.	585	500		550		535		933	617	1.6 times (+348)
2.	"	564		456		468	588	468			1.0 times (+ 24)
3.	0.3 cc. "	200	373		213		350		320		1.9 times (+173)
4.	0.2 cc. infusion (in 100 cc. of phys. saline)	130			320					280	2.5 times (+282)
5.	0.5 cc. infusion (in 200 cc. of Ringer solut.	250	392		541		350			625	2.5 times (+282)
6.	ACTH 10 mg. infusion (in 200 cc. of phys. saline)	467			733		300			1050	2.3 times (+583)
	Control-Experiment										
7.	Spalon 2 mg. intraven.	564		486			486			564	1.0 times (+ 0)
8.	Spalon 4 mg. intraven.	317	233		300		283				0.9 times (− 17)
9.	Physiol. saline solut. 0.3cc. (directly in l. adrenal cortex)	242	233					250			1.0 times (+ 8)

to 373 γ/10 cc. blood in five minutes (No. 3). The chemocorticoid substance was not increased (from 564 γ to 588 γ) in No. 2, in which the blood flow coming from the direction of the diaphragm had not been checked by venous ligation exceptionally; the result is presumably due to the fact that the blood from the adrenals may be diluted by far greater amount of blood flow running down from the direction of the diaphragm.

In the No. 4 and No. 5 cases, the epinephrine (0.2–0.5 cc. mixed respectively in 100 cc. and 200 cc. of the physiologic saline solution was infused into the femoral vein for an hour.

In these cases the chemocorticoid was increased from 130 γ to 320 γ in 15 minutes (No. 4, 2.5 times) and from 250 γ to 625 γ in 60 minutes (No. 5, 2.5 times), after the epinephrine-infusion. The chemocorticoid increase through the same dose of epinephrine seems to be greater in the cases of infusion than those of rapid injection.

In the No. 7 case, the dose of 10 mg. ACTH dissolved in 200 cc. of physiologic saline solution was infused intravenously for one hour. The chemocorticoid was raised from 467 γ to 1050 γ in one hour (2.3 times). The rate of increase in the case of ACTH-infusion is almost the

same as in the cases of the epinephrine infusion contrary to our expectation.

The control experiment was performed in the No. 8, 9 and 10 cases. Namely, 2 and 4 mg. of Spalon (Neocyanocor:photosensitizing drug) in 5 and 20 cc. of distilled water was injected into the femoral vein or 0.3 cc. of physiologic saline solution was injected directly in the left adrenal cortex. The chemocorticoid content in the adrenal vein blood remained almost unchanged after the injection (from 564 γ to 564 γ and from 317 γ to 300 γ, and from 242 γ to 250 γ).

From these findings it is almost certain that the epinephrine-injection causes an increase of chemocorticoid substance in the adrenal venous blood, suggesting probably the increased hormonal secretion from the adrenal cortex.

B. Experiments on the Hypophysectomized Dogs

Epinephrine (0.3–0.5 cc. of 0.1% solution) was similarly injected on the hypophysectomized dogs between 1 and 7 days after the extirpation of the pituitary glands and the chemocorticoid substance was measured in the adrenal venous blood in these animals.

The results are given in Table III. As this Table indicates, the chemocorticoid content was increased after the epinephrine-injection, the average rate of increase amounting to 1.9 times as much as before the injection. The table shows also that the rate of increase of chemocorticoid by the epinephrine injection shows a tendency of gradual decrease, beginning from the third day after the extirpation of hypophysis. The former level of the chemocorticoid before the epinephrine injection tends also to reduce gradually from the third day after the operation, particularly, it is on the lowest level on the 7th and 8th days, suggesting that the adrenal cortex may beginn to undergo atrophic change approximately from the third day after the hypophysectomy.

In the No. 8 case in Table III, the rise of the chemocorticoid was especially remarkable after the ACTH-injection (from 18 γ/10 cc. to 72 γ/10 cc.—4 times), as compared with the case of epinephrine-injection, though the former level was very low.

Discussion

The humoral control over the adrenal cortex by the pituitary gland (through ACTH-liberation) has been generally accepted. Concerning the mechanism of the ACTH-liberation from the anterior pituitary glands acting on the adrenal cortex there are several theories (epinephrine theory of Long,[5] peripheral-humoral mechanism of Sayers[6]). According to Long, an organism, confronted by stress or, mobilized epinephrine from its adrenal medulla as an emergency reaction by Cannon. Thus secreted epinephrine promotes the ACTH-release by acting on the anterior pituitary

TABLE III

Chemocorticoid Content in the Left Adrenal Vein in Hypophysectomized Dogs. Effect of Intravenous Injection of Epinephrine

(γ per 100 cc. blood)

No.	Method of Injection	Days after extirpation \ Time (min.)	0	5	10	15	20	25	30	40	45	Rate of increase
1.	0.4 cc. 0.1% Epinephrine Intraven.	1	384	880	320			600			376	2.3 times (+496)
2.	0.3 cc. "	2	174	186		207		252			318	1.8 times (+144)
3.	0.5 cc. "	3	112	120	168		300		336			3.0 times (+288)
4.	0.3 cc. "	3	300	414				414		564		1.9 times (+264)
5.	0.5 cc. "	3	387	477				372				1.2 times (+ 90)
6.	0.5 cc. "	4	170	270	250		230		300	260		1.8 times (+130)
7.	0.3 cc. "	7	116	136		96			112		100	1.2 times (+ 20)
8.	ACTH 10 mg.	8	18	54		30			72		30	4.0 times (+ 54)

gland by means of humoral way, which in turn causes the secretion of adrenocortical hormone. The reason of adopting epinephrine instead of ACTH in the Thorn's test bases its argument on this Long's theory.

In respect to the epinephrine action upon the ACTH-mobilization from the anterior pituitary, there have appeared already many experimental investigations (Vogt[7] 1947, Paschkis[8] 1950, Corcoran and Page[9] 1948, Hechter[10] 1949). But, we think that there have been no definite findings which gave a conclusive proof to that problem.

Vogt and Pickford[11] (1951) found later that the epinephrine secretion from the isolated perfused adrenal by the blood obtained from the hypophysectomized dog still continued its cortical hormone secrection and that the adrenal venous blood of the hypophysectomized dog frequently assumed towards the adrenalectomized rats toxic properties, which disappeared when epinephrine was being infused. She had said " whether this phenomenon is the expression of a secretion of cortical hormone produced by the adrenaline cannot at present be decided. Nevertheless, it favours the view that adrenaline, besides releasing ACTH, can also act on the adrenal cortex by some other means." Speiers[12] (1949) also reported that the secretion from the adrenal cortex did not entirely dis-

appear still after the hypophysectomy, and Deane and Greep[4] (1946) had an opinion that the mineralo-corticoid might be secreted automatically, independently from the ACTH-control.

Vogt suggested, in respect to the mechanism of the adrenocortical hormonal secretion through the epinephrine, that the possibility of direct stimulation of the adrenal cortex through the epinephrine might exist to some extent, besides the indirect stimulation through the ACTH mobilization from the hypophysis.

Independently from Vogt, Hungerford[13] (1949) found the decrease of peripheral circulating lymphocytes after the epinephrine-injection either in the normal, demedullated or hypophysectomized rat, while he could'nt observe any decrease of lymphocytes in the total adrenalectomized rat. And he pointed out the possibility that the epinephrine might have partly a direct influence without the aid of hypophysis on the adrenal cortex. Kessel[14] (1936) and Crile[15] (1937) had reported suggestive clinical cases: both cases of Cushing's disease (pituitary basophil adenoma) observed in females aged 17 and 18 had improved remarkably after the section of the N. splanchnicus on both sides.

We could confirm in these series of experiments that the stimulation of the N. splanchnicus caused a remarkable increase of chemocorticoid substance in the adrenal venous blood only on the stimulated side and further made sure that this increase of the chemocorticoid through the stimulation of the splanchnic nerve could be found still in the hypophysectomized dog. We could obtain also histological findings supporting the above view.

The finding that the small amount of epinephrine injected intravenously caused an increase of chemocorticoid in the adrenal venous blood, even in the hypophysectomized animal, also offers an important contribution to this problem. It favours the view that the epinephrine or the splanchnic nerve may act on the adrenal cortex by some peripheral mechanims, besides releasing ACTH. The detail of the secretory mechanism by the splanchnic nerve must be inquired more closely, but it may be said, at least, that the adreno-cortical secretion is regulated not exclusively through the ACTH from the anterior pituitary gland, but it is controlled directly through the N. splanchnicus or through some other peripheral mechanism. Whether the epinephrine stimulates adrenocortical cell directly, or it acts on the cell through the excitation of the splanchnic nerve or through other ways, it is not easily decided. But, at any rate, we think it may be an undeniable fact that the secretion of the cortical hormone is regulated, besides ACTH action from the hypophysis, through some peripheral mechanism, i. e. by the direct or indirect nerve control, by the epinephrine influence, or by the co-operation of both.

There have been two great hypotheses in respect to the defense mechanism of the body against the stress, namely, the emergency function of the sympathico-adrenomedullar system of Cannon and the function of adaptation of the pituitary-adrenocortical system of Selye. As to the defense mechanism of the body, Cannon layed emphasis on the autonomic nervous system, particularly on the sympathetic system, while Selye attached importance to the hormonal system, above all, to the pituitary-adrenal system. According to Sayers,[6] the sympathico-adrenal system of Cannon plays the role of ignition to the defense function of the organism in emergency, and the pituitary-adrenal system promotes the universal defence activity of the organism by mobilization of the hormonal system.

At any rate it is certain that the autonomic nervous system and the hormonal system work in co-operation with each other in respect to the defense activity of the body against stress.

We believe that the autonomic nervous system and the hormonal system have much more close connection than they are generally considered, and we offer a new concept through our studies, suggesting a close relation between the N. splanchnicus and the adrenocortical system, which has not been supposed hitherto generally. We propose here that the N. splanchnicus-adrenocortical system may play an important role in the defense mechanism of the body.

We want to continue our study in order to confirm in different methods whether the mobilized chemocorticoid substance in the adrenal venous blood through the stimulation of the N. splanchnicus is a real hormone substance secreted from the adrenal cortex or not. We have already demonstrated that the secreted chemocorticoid substance in the adrenal venous blood is not epinephrine itself.

These findings revealed that the theoretical basis for the interpretation of the so-called Thorn's test using epinephrine must be sceptically reexamined, because there are no reliable proof that the epinephrine promotes the secretion of ACTH from the anterior pituitary, and because we demonstrated that the epinephrine-injection resulted in the mobilization of chemocorticoid in the adrenal venous blood even in the hypophysectomized animal. On the other hand, Thorn's test using epinephrine seems to get a new theoretical basis, because it was demonstrated in our experiment that the chemocorticoid substance in the adrenal venous blood is obviously increased after the epinephrine-injection. But, the true mechanism of the transient reduction of blood eosinophils after the epinephrine-injection in the Thorn's test remains still unsolved. The role of the adrenal cortex in reducing blood eosinophils in Thorn's test is not yet settled, because it has been reported that the decrease of eosinophils after the epinephrine-injection was sometimes observed even in

the adrenalectomized persons or in Addison's disease[16][17][18][19][20]. The diagnostic value of Thorn's test in determining the function of the pituitary adrenal system and its theoretical basis demands further investigation.

Conclusion

1. The stimulation of the N. splanchnicus causes a remarkable increase of chemocorticoid in the adrenal venous blood on the stimulated side and this phenomenon is similarly observed in the hypophysectomized animal.

2. The histological investigation of the adrenal cortex after the stimulation of the splanchnic nerve reveals the hyperactivity of cells in the cortical layers, just in accordance with the increased chemocorticoid in the adrenal venous blood.

3. The adrenocortical layers after the section of the splanchnic nerve show the histological features, indicating reduced secretory activity.

4. The intravenous injection or infusion of a small amount of epinephrine causes a marked increase of chemocorticoid in the adrenal venous blood and this is also observed in the hypophysectomized animal.

5. In respect to the secretory mechanism of the adrenocortical hormone, we presume there might be a direct neural control on the adrenal cortex through the N. splanchnicus or some other peripheral mechanism without the aid of the pituitary gland, besides the pure humoral control through ACTH. Namely, the fact that the chemocorticoid substance is mobilized in the hypophysectomized animal either by the stimulation of the N. splanchnicus or by the epinephrine-injection, suggests the possibility that the secretory mechanism of adrenal cortex may be under the control of the peripheral neurohumoral mechanism, besides under the ACTH through the anterior pituitary gland.

6. The theoretical basis for the so-called Thorn's test using epinephrine instead of original ACTH must be reexamined, because there is a new fact that the epinephrine injection produces an increased chemocorticoid in the adrenal venous blood in the hypophysectomized animal and that the reduction of circulating eosinophils after epinephrine-injection may occur through some other mechanism.

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Intracardiac Phonocardiography

Second Report

By

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In the previous report[1], the authors described the method of intracardiac phonocardiography, on which several improvements were later made in order to assure more precise analyses and these are described in the present paper.

A fine, shielded wire was used for the lead wire between the metal stick at the tip of the catheter and the grid of the oscillator circuit. This limited the conversion of frequency by high frequency to the tip of the catheter. By this means, it became possible to record more selectively the vibration in the desired portion of the heart cavity.

By a similar method, Tomomatsu and Takasaki[2],[4] obtained the slow vibration from the right auricle. However, they did not use a shielded wire for the lead and their apparatus is said to be entirely insensible to sound waves when the catheter left in the air.

The apparatus used in the present experiment, as long as the tuning was perfect, is extremely sensitive to sound waves when the catheter is left in the air, showing that the catheter could act as an extremely small microphone which could not have been manufactured to date.

It follows, therefore, that the apparatus and the object of research by Tomomatsu and Takasaki are a great deal different from those of the present study.

Experimental

Using over 20 adult dogs, the catheter was introduced into right heart by incision through the cervical vein, or to the left heart through the carotid artery. The position of the tip of the catheter was confirmed by the fluoroscope.

Venous catheterisation was also carried out on three human beings

Fig. 1. - One catheter in left descending limb of pulmonary artery, and another in left ventricle.

with this catheter microphone, in one of which the catheter reached the pulmonary artery and heart sounds at various parts in the heart was recorded on the tape-recorder.

In continuation of the previous report, results obtained with dogs are described in the following.

1. Fig. 2a is the recording obtained from the descending limb of the left pulmonary artery, and b, that from the common pulmonary artery. Both are high-pitched murmur of holosystole and the contour is oval or ellipsoidal, similar to the murmur of semilunar valve stenosis. The time elapsed from Q in the Ecg. to the beginning of murmur is 0.03 seconds shorter in b than in a. This means that the record obtained is that of a whirlpool of blood flow at the tip of the catheter.

2. Fig. 3a shows the recording obtained by contacting the tip of the catheter on the inner wall of the left ventricle by its introduction through the carotid artery, and b, the recording from the chest wall made at the same time. The former recording shows the distinct auricular sound. The first sound complex of which is very similar to the first sound from the chest wall.

3. Figs. 4, a, b and c are the recordings from various points in left ventricle of the same dog as above. Presystolic ellipsoidal murmur is

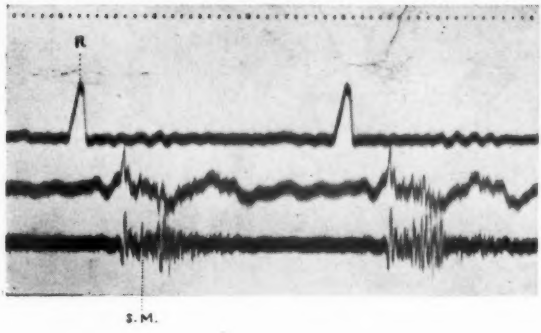

Fig. 2 a

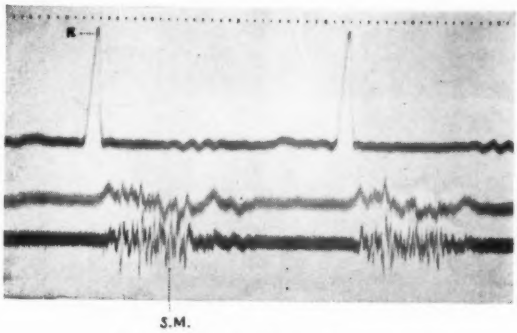

Fig. 2 b

Fig. 2. Tracings from pulmonary artery.

especially well shown in Fig. 4a. It is not clear whether this resulted from a temporary mitral stenosis caused by the tip of the catheter having pushed the mitral valve, or whether such a whirlpool of blood flow exists in the left ventricle. This presystolic murmur cannot be observed from chest wall at the same time.

4. Fig. 5 is the recording obtained from a point near the aortic valve in the left ventricle. It shows the auricular sound and a murmur of small amplitude that follows it. The first sound is either prolonged or split, and follows a rough systolic murmur. The second sound is in some cases split and a large third sound follows.

5. Fig. 6a and b. When the tip of the catheter is placed in the blood stream in aortic arch, holosystolic, high-pitched murmur is present, as in the case of pulmonary artery, and it is very similar to the auscultatory findings of aortic stenosis. When the tip of the catheter is contacted with aortic wall, the first and second sounds become more clear, as can be seen from Fig. 6b. In this instance, this strong systolic murmur can-

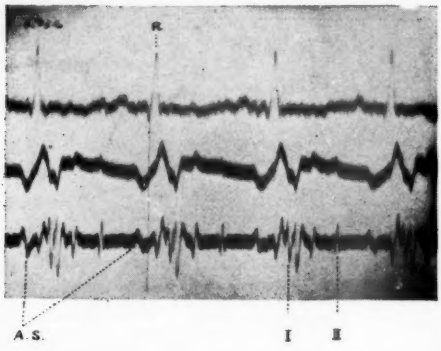

Fig. 3 a

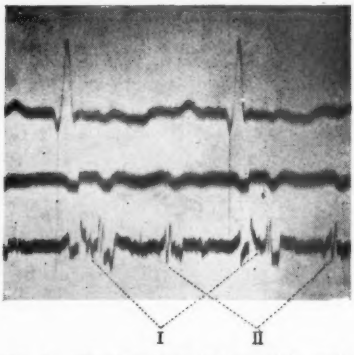

Fig. 3 b

Fig. 3. Tracings from left ventricle and chest wall.

not be seen in the cardiograph taken from chest wall at the same time (Fig. 6c). The whining of a dog is extremely damaging to the recording from chest wall, but it does not give so much effect on the cardiograph taken from the left ventricle or aorta. These results show that myocardium and aortic wall exert an extreme damping effect on vibration, especially of higher frequency.

Summary

1. Intracardiac heart sounds of dogs and men were recorded on a tape-recorder and these recordings were heard by many cardiologists at the Annual Meeting of the Society of Angiocardiology of Japan in 1953.

2. The data obtained to date by the present authors indicate that there exists a certain amount of blood whirlpool inside the heart cavities and arteries. Although this whirlpool is mostly in audible range, the

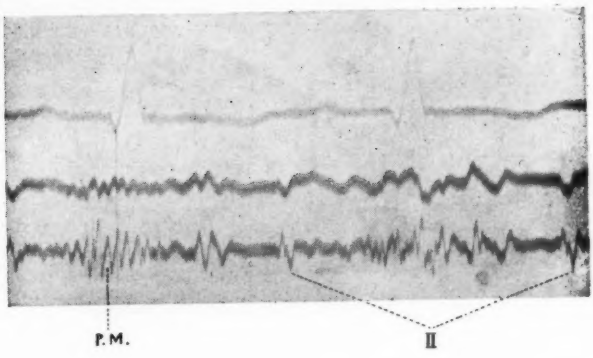

Fig. 4 a

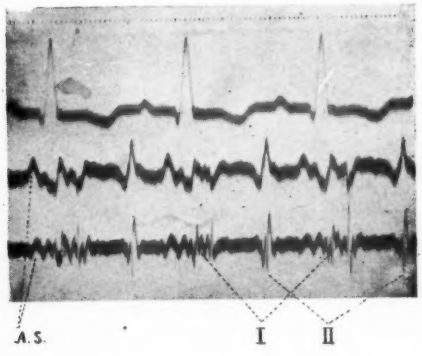

Fig. 4 b

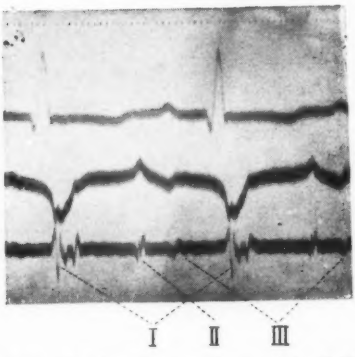

Fig. 4 c

Fig. 4. Tracings from various points of left ventricle.

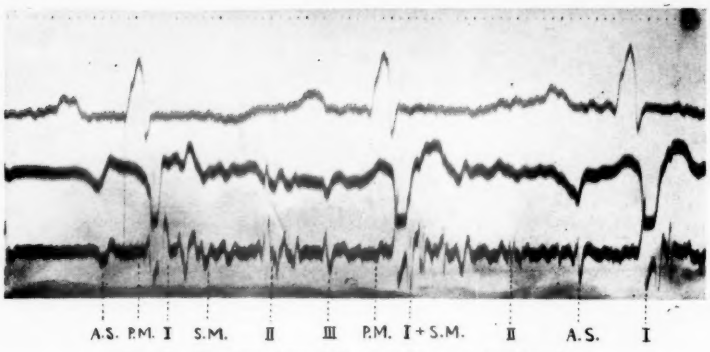

Fig. 5. Tracings from left ventricle near aortic valve.

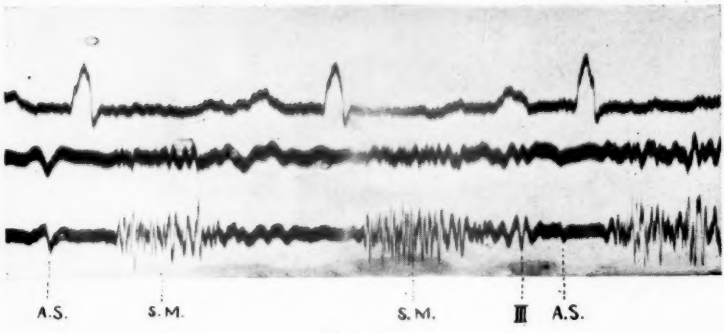

Fig. 6 a

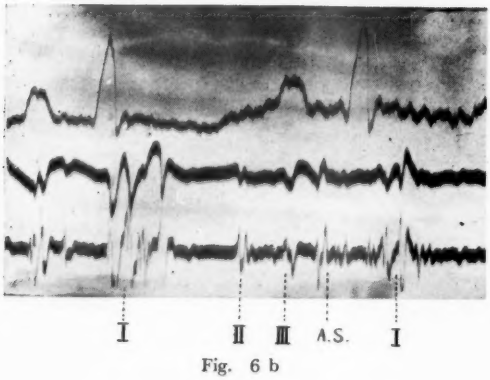

Fig. 6 b

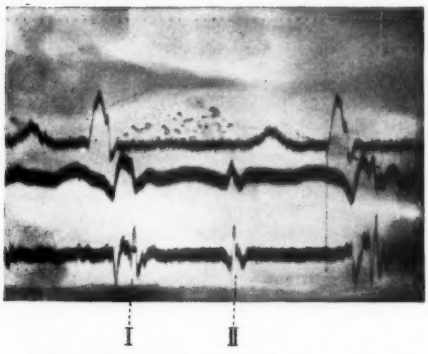

Fig. 6 c

Fig. 6. Tracings from aortic arch and chest wall.

damping effect of heart and arterial walls makes it impossible to hear these sounds upon chest wall in normal healthy case.

3. When the catheter tip is placed in blood stream, chiefly course sounds due to blood whirlpool is recorded, and when the tip is contacted with the inner wall of the heart, chiefly vibrations of a solid structure is recorded. This latter is thought to be one similar to heart sounds obtained from chest wall.

The authors take this opportunity to express their gratitude to Prof. Tasaka for the reviewing of this manuscript.

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Congenital Gigantism of Peroxidase Granules*

The First Case ever Reported of Qualitative Abnormity of Peroxidase

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The present case is, I believe, worthy of report because of a *monstrous* malformation of peroxidase granules of blood leucocytes. The peroxidase reaction I used is that sensitive method of Sato-Sekiya's peroxidase stain.[1] In spite of the fact that this method is now widely used, —many books on hematology and diagnostics recommend the use of it,—such a case has not been reported within my knowledge. I presume the present case will be the very first case ever reported of *qualitative* peroxidase abnormity.

Case Report

T. K. an 11 month old Japanese male infant, was admitted to the Tohoku University Hospital on December 2, 1950, because of an abdominal distention and an acute upper respiratory tract infection.

Explanation to the pedigree of the present case (Cf. Fig. 1): Parents are of consanguinous marriage. One elder brother (Fig. 1, V_1) died in the second year of life and two sisters (Fig. 1, V_3 and V_6) in the third year during some infection. Whether these three cases showed an abnormity of peroxidase granules is unfortunately unknown. But these brothers and sister had in common with the present case: photophobia, pale color of the hair, pigmentation of uncovered parts of skin, frequent occurrence of pustules with generalized lymphadenopathy and marked abdominal distention with hepatosplenomegaly.

History of the present case: The mother's pregnancy was uncomplicated. The patient was born at term with normal delivery. The weight was 2800 g.. At birth the pale color of the hair and the skin was noticed. Until five months of age, besides photophobia, dark pigmentation was noticed on the face and the back of hands and feet and simultaneously a more or less darker pigmentation of hair was noticed. His growth and development were

* Paper read in the 51st Meeting of the Miyagi Prefectural Pediatric Society, Sept. 11, 1953.

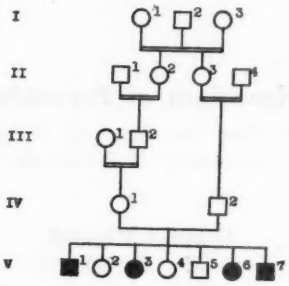


Fig. 1. The pedigree of the present case. Explanation to the pedigree:
V_1, V_3, V_6 . . . . . fatal before full 3 years of age.
V_7 . . . . . the present case.
The pedigree is reliable only as to V. As for I–IV, the mother of the present case did not know the details, so that it is probable that in each of the generations I–IV there was one or some infants who had died young.

rather retarded, as he was able to sit by himself only at nine months of age. He was 11 months old when he was admitted to our Hospital and then he could neither creep nor stand even with support. At 7 months of age, he had pustular lesions on the trunk with formation of ulcers and scars and with concomitant enlargement of superficial lymphnodes. During the time the abdomen had been gradually distended. Five days prior to admission, he had a high temperature up to 38°C, mild cough, loss of appetite and became distinctly prostrated. The mother was afraid that the child might die of the present illness, because she had already experienced the death of three other children of hers who had succumbed to a similar affection, and she brought the present case to the Hospital in the hope that something would be done to save him from death.

Physical examination: The physical examination upon admission revealed a fairly well developed and seriously ill infant. Measurements were 8640 g. in weight (normally 8920 g.), and 45.5 cm, 47 cm and 44.7 cm in circumferences of head, chest and abdomen respectively. His facial expression was mask-like. The hair of his head and eyebrows were whitish grey. The diagnosis of xerodermia pigmentosum was confirmed by Dr. M. Ito, Professor of Dermatology. The brown pigmentation was noticeable in the peripheral part of the face, especially distinct in subauricular regions. The mucous membrane of the upper lid appeared pale. The buccal membrane was free from pigmentation. The tongue seemed to be rather too large. The dark pigmentation of the skin was noticed on the extensor side of the four extremities too. The ophthalmoscopic examination revealed the presence of albinismus fundi oculi utrisque (Prof. Y. Hayashi of Ophthalmology). The skin was sweating, but not itching. There was a generalized lymphadenopathy (cervical ##, cubital +, occipital + and inguinal +) and the coccygeal region was eczematous. Lungs and heart were clear. The pulmonic second sound was

accentuated. The pulse was 102 and the respiration 44 per minute. The blood pressure was 120 systolic and 70 diastoric. The abdomen was moderately distended. The fluctuation was negative, while flanks were dull on percussion. The liver was palpable 2 fingers breadth below the right costal margin, with smooth surface and firm consistency. The spleen was remarkably enlarged, extending up to two fingers breadth below the umbilicus, with smooth surface and firm consistency. The deep tendon reflexes were normal.

Laboratory findings upon admission: The stool was guaiac negative. The urobilinogen was slightly positive in urine. The X-ray of long bones showed epiphyseal transversal lines. The X-ray of the skull and of the hip joints were negative.

Course of illness (Cf. Fig. 2. Table I. Table II): During hospitalization, he ran irregular fever up to 39°C, as is shown in Fig. 2. From the second hospital day on, a daily dose of 200,000 units of penicillin was given intramuscularly for ten days without effect.

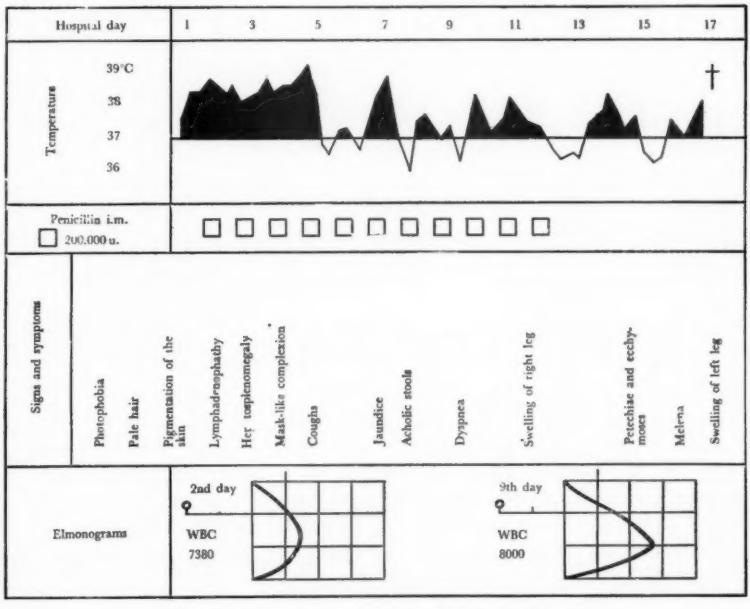


Fig. 2. The course of illness of the present case.

On the second hospital day, blood examination revealed a marked neutropenia with the characteristic abnormity of peroxidase granules of leucocytes which will be described in detail in the following chapter. RBC was 4,100,000, Hgb. 10.8 g/dl, platelets 180,000, bleeding time 3 minutes and Rumpel-Leede's phenomenon was positive. On the 5th day, icterus index of blood serum was 13, v.d. Bergh prompt direct positive. Takata-Ara reaction ##.

TABLE I

Blood Counts of the Present Case

Date 1950		Dec. 3	10	18
Hospital day		2	9	17
WBC*	/cmm	7380	8000	14300
Basophils	/cmm	0	0	0
Eosinophils	/cmm	0	0	0
Lymphocytes	/cmm	6130	6560	4720
Monocytes	/cmm	590	1160	1070
Neutrophils	/cmm	660	280	8500
Juvenile forms	%	35	.....	4
Band forms	%	45	30	15
2 segmented forms	%	20	45	40
3 segmented forms	%	0	25	31
4 segmented forms	%	0	0	9
5 segmented forms	%	0	0	1
Nucleated RBC	/cmm	present	present	1680
RBC	millions/cmm	4.10	3.40	2.20
Hgb	g/dl	10.8	8.3	5.5
Color index		0.87	0.81	0.83
Platelets	thousands/cmm	180	.....	36
Bleeding time	minutes	3	(120)	.....
Coagulation time	minutes	4~9	.....	.....
Rumpel Leede's phenomenon		positive	.....	.....

* The differential leucocyte picture was shown by way of Elmonogram (Cf. Fig. 2). The Elmonogram was devised in 1947 by Sato and Hayashi.[6]

On the 7th hospital day, bleeding time was prolonged to 2 hours, and he became anemic and dyspneic. On the same day, he developed jaundice with acholic stools and bilirubinuria.

On the 9th day, RBC was 3,400,000, Hgb. 8.3 g/dl, WBC 8,000/cmm neutrophils 280/cmm. The sternal puncture was performed. The myelogram did not show any leukemic proliferation of single type of cell (Cf. Table II), but the qualitative abnormity of peroxidase granules of leucocyte precursors was the most outstanding characteristic feature.

On the 13th day, a marked swelling of right lower leg with tenderness but without redness and local heat was noticed, and it extended to the thigh in the following two days.

On the 15th day, petechiae and ecchymoses appeared on the right flank and they increased in number and spread far and wide on the entire body surface in the following days.

On the 16th day, he passed two tarry stools. The fontanel was sunken and the oral membrane was dry. He was more anemic and went progressively

TABLE II

Bone Marrow Picture of the Present Case

I.	Total nucleated cell count	28800 per cmm.
II.	Erythropoiesis	
	Proerythroblasts	2.8%
	Normoblasts	58.0%
	Mitotic forms	0.1%
III.	Leucopoiesis	
	Neutrophils	
	Myeloblasts	4.3%
	Early myelocytes	4.1%
	Intermediate myelocytes	10.1%
	Late myelocytes	8.0%
	Bands	0.8%
	2 segmented forms	0.2%
	Eosinophils	
	Myeloblasts	0.2%
	Early myelocytes	0.7%
	Intermediate myelocytes	0.4%
	Late myelocytes	0.5%
	Bands	2.3%
	2 segmented	1.7%
	Monocytes	
	Monoblasts	0.4%
	Monocytes	0.1%
IV.	Reticuloendothelial cells	
	Lymphoid reticulum cells	1.8%
	Lymphocytes	2.9%
	Plasmacellular reticulum cells	0 %
	Plasma cells	0.1%
	Mitotic forms	0.1%
V.	Thrombopoiesis	
	Megakaryoblasts	0.3%
	Megakaryocytes	0.1%
VI.	Myeloid-Erythroid ratio	0.7:1.0

downhill.

On the 17th day, the left leg was swollen as well as the right. RBC was 2,200,000, Hgb 5.5 g/dl, WBC 14,300/cmm, neutrophils 8500/cmm (terminal leucocytosis) and platelets 36,000/cmm. He became apathetic and died.

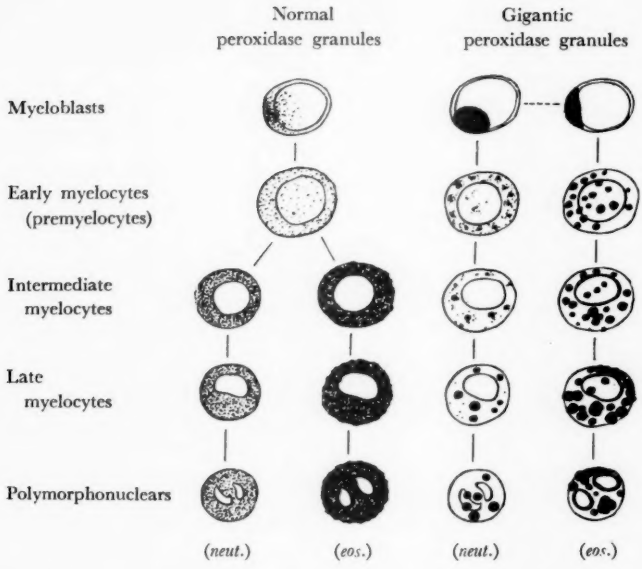
Blood Pictures of the Present Case

Method: The blood or/and bone marrow specimens were stained by the following three methods: Sato-Sekiya's peroxidase method, the Giemsa method and the supravital method with neutral red respectively.

Results: I. Peroxidase stain (Cf. Fig. 3. Fig. 4 and Fig. 5.)

1) Neutrophils: Under Sato-Sekiya's peroxidase stain method, the cytoplasm of normal neutrophils is, as is well known ,entirely filled with fine peroxidase granules. The neutrophils of the present case, however, have scarcely any peroxidase granules except in some limited parts of

Fig. 3



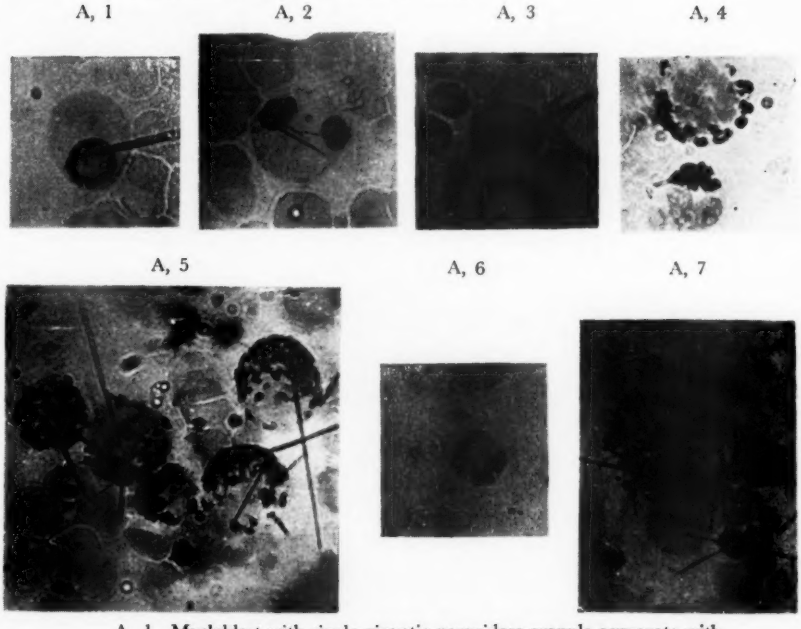
their cytoplasm, while almost all peroxidase granules aggregate very compactly, each group forming a colossal mass, "Gigantic Peroxidase Granule" (G.P.G.). This is spherical or oval, 1 to 3 μ in diameter, or rarely spindle-like in shape, appearing intensely blue and is contained in one cell in the number of 1 to 7. Some of the G.P.G. may coalesce to each other. There may be among the G.P.G. in the cytoplasm a few safranophilic brownish red granules, which give no peroxidase reaction.

2) Eosinophils: The eosinophils were almost absent in the peripheral blood and were present in the aspirated bone marrow. In the cytoplasm of normal eosinophils, the coarse peroxidase granules are themselves groups of granules, each group appearing to be one coarse peroxidase granule, but they do not make large compact masses. In the present case, however, the cytoplasm is filled with ball-like masses of peroxidase granules i.e. the G.P.G. of various size, 3 to 4 μ in diameter. And the cellular outline seems to be formed by a chain of the G.P.G..

3) Monocytes: Normally the peroxidase granules of monocytes are smaller in size than those of neutrophils. But, in the present case, the overwhelming majority of monocytes have in the cytoplasm the G.P.G. very similar to those of neutrophils described above.

4) Lymphocytes: They show no peroxidase granules. It should also be noticed that there is not any safranophilic granule in their cytoplasm.

Fig. 4 (A). Explanation to all the pictures A.
G.P.G.(s).....gigantic peroxidase granule(s)



A, 1., Myeloblast with single gigantic peroxidase granule aggregate with a peroxidatic needle. In fresh specimens, the entire round field of such G.P.G. would be entirely filled with fine blue peroxidase granules. The nucleus is indented due to the presence of the G.P.G..

A, 2., Myeloblast with two G.P.G.s with needle formation. One G.P.G. with needle formation.

A, 3., " Eosinophilic " myeloblast. In this cell peroxidase granules are aggregated, filling the entire space of cytoplasm together and looking crescent-shaped. Needle formation is seen.

A, 4., Myelocyte (upper) in mitosis with a chain of G.P.G.s and neutrophilic metamyelocyte (lower) with clumping of G.P.G.s.

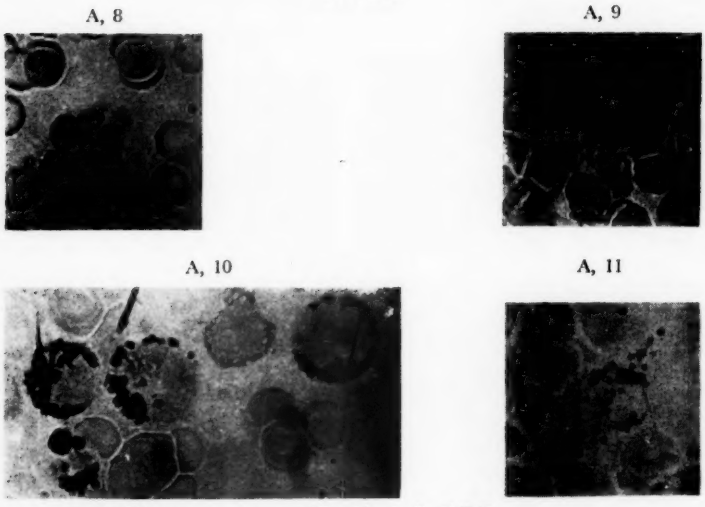
A, 5., Myelocytes, probably neutrophilic, containing numerous G.P.G.s instead of normal-sized fine peroxidase granules. Differentiation between neutrophils and eosinophils is difficult in these cells.

A, 6., Polymorphonuclear neutrophil containing about 10 G.P.G.s in cytoplasm.

A, 7., Early myelocyte (upper) with numerous G.P.G.s covering a part of nucleus. Eosinophilic metamyelocyte (lower) with several G.P.G.s.

5) Neutrophilic myelocytes: The late and intermediate forms of the cells showed a definite inclination to develop the G.P.G. in their cytoplasm, while each of these G.P.G. is smaller and less characteristic

Fig. 4 (A)



A, 8., Monocyte or neutrophil with single G.P.G..

A, 9., Monocyte with several G.P.G.s.

A, 10., Three early myelocytes (upper) with peripheral G.P.G.s. Probably eosinophil metamyelocyte with G.P.G.s filling two corners.

A, 11., Reticulum cell (star-shaped) with many G.P.G.s. (Normally peroxidase-negative. Even if peroxidase-positive in pathologic cases, peroxidase granules would be only small.)

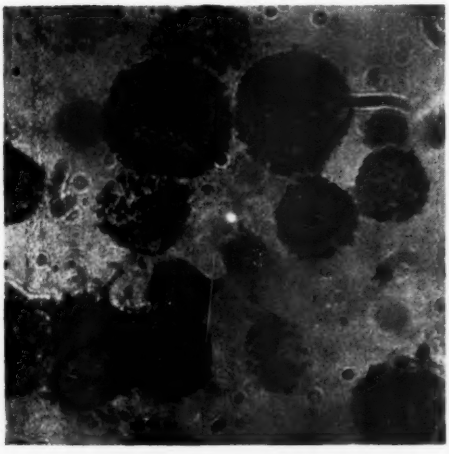
than that of polymorphonuclear neutrophils. There are also a moderate number of intermediate forms between normal sized (or small) peroxidase granule and the G.P.G.. In the early myelocytes, there are typical G.P.G. which are larger in size, less in one cell than those of polymorphonuclears.

6) Eosinophilic Myelocytes: The majority of the cells of late, intermediate and early stages are loaded with the G.P.G. which are more spherical, and plumper than those of neutrophilic myelocytes and filling the most part of the cytoplasm, covering the nuclei partly.

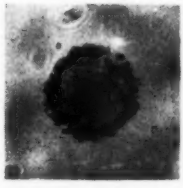
7) Myeloblasts: In a normal myeloblast, most of the peroxidase granules in the cell are only on one side or in one corner (or in two oppsite corners) of the cell, and from such a corner a more or less small number of the peroxidase granules seem to be located radiating toward the equatorial part of the cell. In the present case, the localization of peroxidase granules is limited in one corner or in two opposite corners as in the normal case or occasionally in four corneres of the cell, while, the granules aggregate more or less densely to build a round colossal

Fig. 4 (B). Explanation to all the pictures B.
These pictures represent normal cases, so G.P.G. does not occur here.

B, 1



B, 2

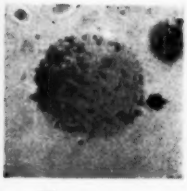


B, 3

B, 4

B, 5

B, 6



B, 1., The bone marrow peroxidase picture of a normal case. The peroxidase granules of these cells are fine and rather diffusely scattered in cytoplasm. One eosinophil myelocyte, which is more heavily loaded with peroxidase granules, is seen in the left lower corner.

B, 2., Neutrophil leucocyte
B, 3., Eosinophil leucocyte
B, 4., Monocyte
B, 5., Early myelocyte
B, 6., Myeloblast

mass, deserving the name of G.P.G. ever more, a veritable monstrous peroxidase mass. The G.P.G. varies in size, mostly 5 to 10 μ and rarely 2 to 3 μ, and the nuclei of some myeloblasts are indented by the presence of the G.P.G.. Some of G.P.G. are found within vacuoles. In some of the myeloblasts, probably "eosinophilic" myeloblasts, peroxidase granules are aggregated, filling the entire space of one corner of the cell and looking crescent shaped. Peroxidase-negative myeloblasts are occasionally seen.

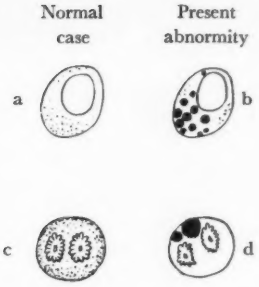


Fig. 5. Gigantic Peroxidase Granules (G.P.G.) in the monocyte and the early myelocyte in mitosis.
a: Normal monocyte, b: Monocyte with G.P.G.s, c: Normal premyelocyte in mitosis, d: Mitotic form of premyelocyte with G.P.G.s.

8) Erythroblasts: All of the erythroblasts examined are apparently peroxidase-negative, but one cell which is peroxidase-positive contained a single spherical G.P.G. in the cytoplasm.

9) Megakaryocytes: All of them examined are peroxidase-negative.

10) Extracellular G.P.G.: They occur very rarely in the blood film and not infrequently in the bone marrow smears, and their size and shape were various as well as those in the cells, suggesting that some of the G.P.G.s could be liberated from the cells of each stage of the cellular development, thus the cells probably subjected to a rapid disintegration.

II. Giemsa stain (Cf. Fig. 6 and Fig. 7)

1) Neutrophils: In most of the neutrophils, there are several round aggregates of the fine violet-pink granules, which look somewhat like small plateletes in appearance. And, among these abnormal aggregates in the cytoplasm, almost none of the specific granules is found to be scattered freely.

2) Monocytes: In the basophilic cytoplasm, there are several round colorless spots, in each of which a group of fine lilac granules occurs as aggregated. Non-aggreagated, free granules are very few in number.

3) Lymphocytes: The cytoplasm contained a large round purplish red or azurophilic body, which was 1 to 3 μ in diameter.

4) Eosinophils: The specific granules are in general, much plumper than normal and different in size in one cell, varying 1 to 4 μ in diameter. These granules are mostly spherical, but some of the largest ones appear rather polymorphous, presumably showing coalescence of two to three original less large, spherical granules. Some of the cells are heavily loaded with these abnormally plump granules.

5) Neutrophilic Myelocytes: The cytoplasm of late myelocytes

Fig. 6

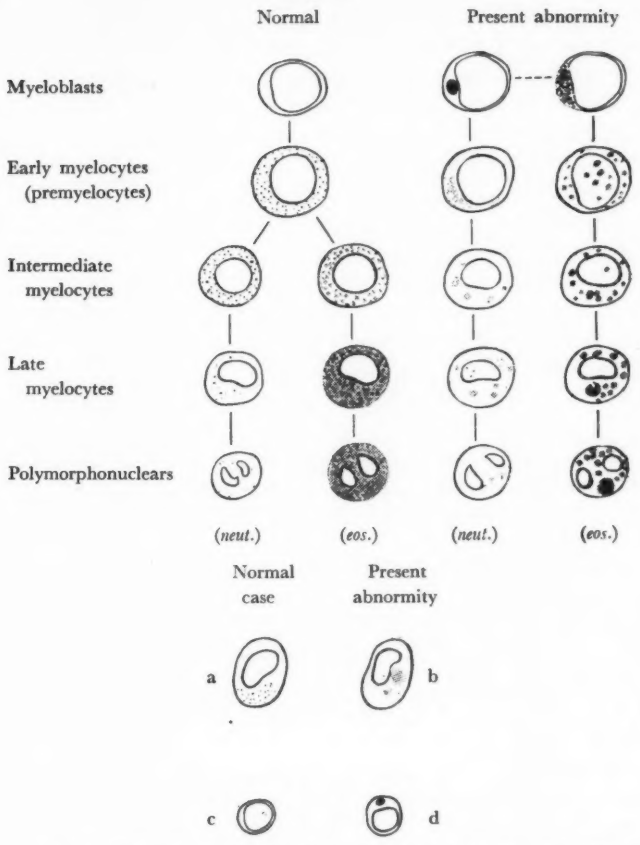


Fig. 7. Abnormal granules in monocyte and lymphocyte (Giemsa stain).
a: Normal monocyte, b: Abnormal monocyte showing clumping of granules, c: Normal lymphocyte, d: Abnormal lymphocyte with a large azurophilic granule.

contained multiple round aggregates of neutrophilic granules almost similar to those found in polymorphonuclears, while the latter is a little more conspicuous. The intermediate myelocytes have a few coarse azurophilic granules, and besides, the cytoplasm shows multiple vacuolization and some of the vacuoles appeared diffusely light reddish yellow or lilac. The early myelocytes (or premyelocytes) have a few fragmented, very coarse granules, appearing purpurish red or azurophilic.

The cytoplasm shows not infrequently marked vacuolization and some of the vacuoles are filled with some material appearing very faintly orange-colored. In some of the cells, the granules are smaller in size and spherical or somewhat needle-like in shape and present in a larger number.

6) Eosinophile myelocytes: They show almost similar abnormity of the specific granules to those of mature eosinophils, while it is noticed that, the apparently younger the cell, the less characteristic is the abnormity of the granules.

7) Myeloblasts: Myeloblasts with " huge azurophilic " granules; they have an extraordinally large round vacuoles in intensely basophilic cytoplasm, which contains round purpurish red granules. These huge azurophilic granules or inclusion bodies are varied in size, 3 to 7 μ, correspondinly to the size of vacuoles, and they are usually round but occasionally appeared modified.

Myeloblasts with eosinophilic granules: They contain definitely eosinophilic granules, which are spherical and less than 1 μ in diameter forming a crescent-shaped space filled with eosinophilic granules in one corner of the cell.

Myeloblasts without granulation are occasionally seen and some of them have large vacuoles only.

8) Neither erythroblasts nor megakaryocytes show particular abnormity, while both of them showed pathologic regeneration. The megakaryocytic picture is identical with that of idiopathic thrombocytopenic purpura, characterized by the presence of immature megakaryocyte containing giant platelet in the strongly basophilic cytoplasm.

In the bone marrow smears, extracelluar eosinophilic granules are found not infrequently.

9) As to nuclear structure, the staining of chromatin is rather weak, esp. in some myeloblasts and early myelocytes, though in a few others chromatin is rather dark stained. In many myeloblasts and early myelocytes there are a larger number of nucleoli, some of which are more or less large and polymorphous.

III. Supravital method (Cf. Fig. 8)

This revealed a peculiar behavior of neutral red vacuoles in neutrophils, monocytes, lymphocytes and red blood corpuscles. Almost immediately after blood smear was prepared, large spherical neutral red vacuoles appeared in the cytoplasm of these cells. In neutrophils and monocytes, the vacuoles are 1 to 3 μ in diameter and occur up to 10 in number in a single cell. In lymphocytes and erythrocytes, vacuoles are less than 2 in number, usually only one in number in a single cell.

IV. The Oxidase Reaction

The oxidase reaction was examined by the Winkler-Schultze method.

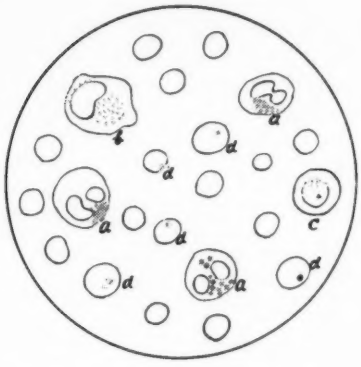


Fig. 8. Peculiar neutral red vacuoles or granules. The peculiar behavior of neutral red vacuoles under the supravital method.
a: Neutrophils showing clumping of neutral red vacuoles in the cytoplasm, b: Monocyte, c: Lymphocyte, d: Erythrocytes.
The neutral red stained vacuoles or granules of erythrocytes of this case were considered to be a characteristic features of the present abnormity manifested in the cells. It is interesting to not that none of the Howell-Jolly bodies was found in the Giemsa stained blood smears of this case.

This, too, revealed a remarkable malformation of oxidase granules of leucocytes, apparently very similar, in size, (group of granules) and localization in cytoplasm in each developmental stage of granulocytes, to those of peroxidase granules i.e. the G.P.G.. In fact, there was no much difference between the oxidase picture and the peroxidase picture except for that each group of oxidase granules showed more clearcut round outline than that of peroxidase granules. I desire to suggest the designnation of Congenital Gigantism of Oxidase Granules for this qualitative abnormity of oxidase granules of leucocytes.

Remarks

The abnormity of different cells under the peroxidase, the Giemsa and the supravital stain methods is summarizingly shown in Table III.

Discussion

A. Sato, the former professor of our Pediatric Clinic, held the chair of Pediatrics from 1917 to 1948. He published his peroxidase reaction in collaboration with S. Sekiya[1] in 1926. But they devised the method already in 1920, so that Sato's work with this peroxidase method continued from this year to 1948—thus as long as almost 30 years. But he had not

TABLE III

The Abnormity of Different Cells under the Peroxidase*, the Giemsa and the Supravital Stain Methods

		Peroxidase stain (Sato-Sekiya's method)		Giemsa stain				Supravital stain with neutral red
		"G.P.G."		aggregates of neutrophilic granules	plumper eosinophilic granules	large azurophilic granules		abnormal vacuoles
		10–5 μ	4–1 μ			4–7 μ	1–3 μ	
Leucon	neutrophils							
	polymorphonuclears	−	++	++	−	−	−	++
	myelocytes	−	+	+	−	−	−	?
	eosinophils							
	polymorphonuclears	−	+	−	++	−	−	?
	myelocytes	−	+	−	+	−	−	?
	basophils	?	?	−	−	?	?	?
	promyelocytes	+	+	+	+	+	+	?
	myeloblasts	++	+	−	(+)	++	+	?
	monocytes	−	+	+	−	−	−	+
	lymphocytes	−	−	−	−	−	+	+
Erythron	erythrocytes	−	−	−	−	−	−	+
	juvenile erythrocytes	−	−	−	−	−	−	+
	normoblasts	−	(+) very rare	−	−	−	−	?
	proerythroblasts	−	−	−	−	−	−	?
Thrombon	platelets	−	−	−	−	−	−	?
	megakaryocytes	−	−	−	−	−	−	?
	promegakaryocytes	−	−	−	−	−	−	?
	megakaryoblasts	−	−	−	−	−	−	?
Extracellular granules		+	+	Uncertain	+	−	+	?

* Oxidase reaction presents the same picture as the peroxidase as to the distribution.

come across such a case as I have described above. From the European continent[2] where the above mentioned peroxidase reaction (under the name of "Sato's oxidase reaction or Sato's peroxidase method") has been much in use and from the American continent[3] where the reaction has been much used under the name of "Sato and Sekiya's stain", there has been no report whatever of such a case as my own.

As to the quantitative abnormity or monstrosity, Sato[8] has already reported repeatedly. (He will write, so I am told, systematically about

the quantitative monstrosity of peroxidase granules). But as for the qualitative monstrosity, my own case seems to be the very first one in the world literature.

As described already, the present case had xerodermia pigmentosum, albinismus fundi oculi utrisque and albinism of hair. So the question that there might be some hereditary relation between the present abnormity of peroxidase granule of leucocytes on one side and pigmentary changes of skin, hair and eye-ground was raised. Then three cases of xerodermia pigmentosum, two cases of retinitis pigmentosa and one case of total albinism were subsequently examined, but there was no abnormity of the peroxidase granules of leucocytes in any of these cases. Consequently, it should be concluded that the simultaneous occurrence of xerodermia pigmentosum and albinism of ocular fundi and hair in this case was probably an accidental coincidence, and an unrecognized factor, which is responsible for the occurrence of G.P.G., was independent of the known factors responsible for above quoted pigmentary changes.

Although the present case showed a considerable similarity to a case of acute leukemia of leucopenic type (Cf. Anemia, splenohapatomegaly, lymphadenopathia, hemorrhagic diathesis and acute fatal course.), it will be easy to distinguish the latter condition, for it is associated with a distinct predominance of a single type of cell in the bone marrow or/and in the blood in leukemic cases.

What was striking especially in the present case was the large number of G.P.G., the obvious aggregation of fine peroxidase blue granules producing gigantic clumpy effects in leucocytes. There has been no reported case of any known blood dyscrasia that showed such a monstrous abnormity of peroxidase granules. I am quite unable to account for this monstrous abnormity of peroxidase granules on any other basis than a congenital chromosomal defect which might have been due to intermarriage.

The function of peroxidase in the leucocyte has as yet not been investigated in detail. According to Agner[4] the verdoperoxidase occurs in high concentration in the leucocytes. It would seem strange if this high content of verdoperoxidase were needed merely for the oxidative conversion in the leucocytes. It is conceivable that it also has a function in connection with the general reaction of the leucocytes to infection and similar states. One function of it is connected with the hematopoiesis, I believe it on the basis of " Peroxidase Response[5] " which I described already in this Journal. In the present case an abnormal or rather monstrous arrangement of peroxidase granules in the leucocytes (which were themselves abnormal) might have a close connection with a short span of life of such a patient.

Notes

1) It is to be noted here that the mother's blood did not show any abnormity of the peroxidase picture. The father failed to come to our Department in spite of our repeated request.

2) It is a great regret that I did not apply my own method of counting chamber method[7] of peroxidase reaction, by means of which a possible abnormity of the erythron might have been revealed. As has already been described in supravital method (Cf. III), some erythrocytes showed abnormal pictures and one erythroblast even a peroxidase abnormity. If I shall ever come across a second case of the disease, I shall make a point of studying the erythrocyte peroxidase by this method.

Summary

1. In this paper the first case ever reported of qualitative abnormity of peroxidase of blood leucocytes was outlined and the name of " Congenital Gigantism of Peroxidase Granules " was suggested for this abnormity.

2. The Congenital Gigantism of Peroxidase Granules represents a monstrous malformation of the peroxidase granules—obvious aggregation of fine peroxidase blue granules producing gigantic clumpy effects in each cell—of all the myeloic leucocytes in the blood or/and bone marrow (Fig. 3, Fig. 4 and Fig. 5).

3. The Giemsa stained picture of the blood or/and marrow smears of this case was also in no way normal, while the morphological change was not always corresponding to the abnormal peroxidase granules (Cf. Fig. 6 and Fig. 7).

4. The oxidase granules of blood and marrow cells of this case showed a monstrous malformation similar to those of peroxidase granules. So the name of " Congenital Gigantism of Oxidase Granules " may be used instead.

5. The supravital method revealed a peculiar behavior of neutral red vacuoles in neutrophils, monocytes, lymphocytes and red blood corpuslces (Cf. Fig. 8).

6. This patient had photophobia, xerodermia pigmentosum, albinismus fundi oculi utrisque and albinism of hair. He died at 11 months of age developing, besides bronchopneumonia, hepatosplenomegaly, jaundice, melena and pancytopenia.

7. The family pedigree of this patient is characteristic. The parents were of consanguinous marriage. Three sibs had the same congenital stigmata i.e. photophobia, pale color of hair, pigmentation of uncovered parts of the skin and died very early in life during some infection in the

clinical conditions very similar to the present case. It is not difficult to suppose that these sibs had " Congenital Gigantism of Peroxidase Granules."

8. The Congenital Gigantism of Peroxidase Granules might have a close connection with a short span of life of the patient.

Conclusions

A case with photophobia, xerodermia pigmentosum-like pigmentation of the skin, albinismus fundi oculi utrisque, albinism of hair and pancytopenia was presented in the present paper. The most characteristic feature is congenital gigantism of peroxidase granules in myeloid leucocytes. It is a very fatal disease. The name of " Congenital Gigan tism of Peroxidase Granules " is suggested for this new clinical entity.

Acknowledgement of their help with this study is made to Dr. Y. Hayashi, Professor of Ophthalmology of our University, and Dr. M. Ito, Professor of Dermatology of our University, who examined this case, to Dr. J. Kuwajima, ophthalmologist and Dr. Y. Katabira, dermatologist whose co-operation made possible a study of patients with pigmentary changes of the eye ground and the skin, to the late Dr. M. Yamasaki, former Professor of Anatomy of our University, who was interested in this case and encouraged me to publish this article through his kind advice and criticism, to Dr. T. Mori, Associate Professor of the department of anatomy of our University, who was also interested in this case and kindly prepared many photomicrographs. It is with pleasre that I express my gratitude to doctors of our Pediatric department, especially Dr. K. Obara, Dr. I. Nakagawa, Dr. K. Ozawa, Dr. K. Matsuura and Dr. Y. Hayashi, who supplied clinical data and assisted me in many things.

I am particularly grateful to Dr. A. Sato, Professor emeritus of our University, for his helpful advice and criticism. The very suggestion that this might be a new clinical entity came from him who had had about 30 years' experience of peroxidase reaction.

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6) Sato & Hayashi, *ditto*, 1947, **49**, 37.

7) Higashi, ditto, 1953, **57**, 367.

8) Sato, the Quantative and Qualitative Monstrosity of Peroxidase Reaction, to be published in a coming issue of this Journal.

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